Exhibit 51

REPORTS

Prospective Study of Talc Use and Ovarian Cancer

Dorota M. Gertig, David J. Hunter, Daniel W. Cramer, Graham A. Colditz, Frank E. Speizer, Walter C. Willett, Susan E. Hankinson

Background: **Perineal talc use has been associated with an increased risk of ovarian cancer in a number of case–control studies; however, this association remains controversial because of limited supporting biologic evidence and the potential for recall bias or selection bias in case–control studies. In this study, we conducted a prospective analysis of perineal talc use and the risk of ovarian cancer.** ***Methods:*** **The Nurses' Health Study is a prospective study of 121 700 female registered nurses in the United States who were aged 30–55 years at enrollment in 1976. Talc use was ascertained in 1982 by use of a self-administered questionnaire: after exclusions, 78 630 women formed the cohort for analysis. Three hundred seven epithelial ovarian cancers subsequently diagnosed in this cohort through June 1, 1996, were confirmed by medical record review and met inclusion criteria. Proportional hazards models by use of pooled logistic regression were used to derive relative risks (RRs) and 95% confidence intervals (CIs).** ***Results:*** **In 1982, 40.4% (n = 31 789) of the cohort reported ever using talc, and 14.5% (n = 11 411) reported ever using talc daily. We observed no overall association with ever talc use and epithelial ovarian cancer (multivariate RR = 1.09; 95% CI = 0.86–1.37) and no increase in risk of ovarian cancer with increasing frequency of use. There was a modest elevation in risk for ever talc use and invasive serous ovarian cancer (multivariate RR = 1.40; 95% CI = 1.02–1.91). The risk of epithelial ovarian cancer for talc users was not greater among women who had never had a tubal ligation (multivariate RR = 0.97; 95% CI = 0.71–1.32).** ***Conclusion:*** **Our results provide little support for any substantial association between perineal talc use and ovarian cancer risk overall; however, perineal talc use may modestly increase the risk of invasive serous ovarian cancer. [J Natl Cancer Inst 2000;92:249–52]**

Talc was originally implicated as a possible ovarian carcinogen because of its chemical similarity to asbestos, which has been linked to ovarian cancer in occupational settings and is associated with mesotheliomas histologically resembling epithelial ovarian cancers *(1–3)*. Perineal use of talcum powder has been positively associated with ovarian cancer risk in a number of case–control studies *(4–13)*, although the magnitude of the associations has been modest, with odds ratios ranging from 1.2 to 1.9, and not all results reached statistical significance *(5,6,8)*. Despite this relative consistency among studies, the limited supporting biologic evidence, together with the possibility of recall and selection bias in case–control studies *(1)*, has raised questions about the plausibility of the association. We, therefore, prospectively examined the relationship between perineal talc use and ovarian cancer risk in a large cohort of U.S. women.

METHODS

The Nurses' Health Study, established in 1976, is a prospective cohort of 121 700 registered nurses living in 11 of the larger states in the United States. Questionnaires were mailed to married, female nurses aged 30–55 years, requesting information on health-related issues, including medical history and potential risk factors for cancer. Follow-up questionnaires have been mailed every 2 years to update information on exposures and to ascertain newly diagnosed diseases. The study was approved by the Human Research Committee at the Brigham and Women's Hospital, Boston, MA.

Ascertainment of cases. We sought medical records from all women who reported a diagnosis of ovarian cancer or who were deceased in each follow-up cycle. Records were reviewed by physicians unaware of exposure status. Histologic subtypes were determined from pathology reports, and epithelial ovarian cancers were classified as serous cancers (including cystadenocarcinoma and papillary adenocarcinoma), mucinous cancers (including adenocarcinoma and mucinous papillary adenocarcinoma), and endometrioid cancers (clear cell and other types, including mixed epithelial tumors). Borderline histologic tumors are included in the analysis. Deaths are reported by relatives and postal authorities, as well as a search of the National Death Index. Mortality follow-up is estimated to be 98% complete in this cohort *(14)*. Cases of epithelial ovarian cancer (International Classification of Diseases Code, ICD183.0), confirmed by medical record review or death certificate, occurring between the return of the 1982 questionnaire and June 1, 1996, were included in the analysis.

Exclusions. Women who did not respond to the question on talc use in 1982 were excluded from this analysis. We also excluded women who had reported a diagnosis of cancer (other than nonmelanoma skin cancer) before 1982, as well as women who reported bilateral oophorectomy, surgery with an unknown number of ovaries removed, and a history of radiation therapy. Validity of self-reported surgical menopause has been assessed previously, and agreement with medical records was more than 97% *(15)*. These exclusions were updated every 2 years. At baseline, 78 630 women were eligible for the analysis. The resulting population after exclusions contributed 984 212 person-years of follow-up and 307 cases of epithelial ovarian cancer.

Ascertainment of talc exposure. Use of talcum powder was ascertained on the 1982 questionnaire in the following ways: "Have you ever commonly used talcum, baby powder, or deodorizing powder *a*) to apply to perineal (private) area? No, daily, one to six times per week, or less than once per week or *b*) to apply on sanitary napkins? No, Yes." We classified "ever talc use" as ever talc use on either the perineal area or sanitary napkins.

Other covariates. Potential risk factors and confounders of the association between ovarian cancer and exposures of interest in this analysis also were obtained from the biennial questionnaires and were updated every 2 years where relevant. Oral contraceptive use was asked every 2 years from 1976 through 1982, by which time use was rare. Tubal ligation history was asked as part of a question on methods of contraception from 1976 through 1984, and, in 1994, women were asked if they had ever

Affiliations of authors: D. M. Gertig, F. E. Speizer, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; D. J. Hunter, G. A. Colditz, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, and Department of Epidemiology, Harvard School of Public Health, Boston, and Harvard Center for Cancer Prevention, Boston; D. W. Cramer, Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital; W. C. Willett, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, and Departments of Epidemiology and Nutrition, Harvard School of Public Health; S. E. Hankinson, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, and Department of Epidemiology, Harvard School of Public Health.

Correspondence to: Dorota M. Gertig, MB.BS., MHSc., ScD., Centre for Genetic Epidemiology, University of Melbourne, 200 Berkeley St., Carlton 3053, Australia (e-mail: Dorota.Gertig@channing.harvard.edu).

See "Notes" following "References."

had a tubal ligation and, if so, at what age. Family history of ovarian cancer was not asked until 1992. Parity was defined as the number of pregnancies lasting 6 months or more and was asked through 1984.

Statistical analysis. Incidence rates (number of cases for each category of exposure divided by person months of follow-up in that cycle) were calculated for each category, adjusting for age in 5-year intervals. Proportional hazards models by use of pooled logistic regression were used to derive relative risks (RRs) and 95% confidence intervals (CIs) of disease for each exposure category *(16)*. For age-adjusted analyses, we categorized variables as follows: parity (0, 1–2, or ⩾3), oral contraceptive use (never, past, or current), tubal ligation (yes or no), postmenopausal hormone use (never, past, or current), cigarette smoking (never, past, or current), and body mass index, i.e., weight in kilograms/height in meters squared (<21, 21.0–22.9, 23.0–24.9, 25.0–28.9, or ⩾29 kg/m^2). In multivariate analyses, we adjusted for age (years) and for potential risk factors by use of indicator variables for each category as described above, except for parity (0, 1–2, 3–4, or ⩾5) and duration of oral contraceptive use (never or <3, 3–5, or >5 years), for which we used a larger number of categories to more appropriately control for confounding. In addition we controlled for age at menarche, duration of breast-feeding, and age at menopause. However, since this did not alter the estimates for talc use, further models did not control for these variables. Body mass index and duration of oral contraceptive use were also entered as continuous variables, and similar estimates were obtained. All RRs reported are multivariate unless otherwise stated. *P* values reported are two-sided.

Results

Three hundred seven women developed ovarian cancer in the cohort from 1982 through 1996 who responded to the 1982 questionnaire on talc use. In 1982, 40.4% (n = 31 789) of the baseline cohort reported ever using talc, of which 14.5% (n = 11 411) were ever daily talc users. Talc use was associated with higher body mass index and inversely associated with current cigarette smoking (Table 1).

We did not observe an overall association with ever use of talc and epithelial ovarian cancer (RR = 1.09; 95% CI = 0.86–1.37). There was also no elevation in risk among daily users of perineal talc, and no trend was seen with increasing frequency of use (Table 2). Talc use on sanitary napkins was inversely related to ovarian cancer, but the association was statistically nonsignificant. Exclusion of use of talc on sanitary napkins from the ever use of talc variable did not substantially alter the results. We also evaluated the risk for women who used both perineal talc and talc on sanitary napkins but did not see an effect compared with never users of talc (RR = 0.90; 95% CI = 0.59–1.37).

When we stratified by histologic subtype, we observed a modest increase in risk for ever talc use for serous invasive cancers (RR = 1.40; 95% CI = 1.02–1.91) but not for all serous cancers (including borderline cancers), endometrioid cancers, or mucinous cancers (Table 3). For women who reported ever daily use of talc, the RR of invasive serous cancer was 1.49 (95% CI = 0.98–2.26). The RRs for ever talc users of less than once per week and one to six times per week were 1.29 (95% CI = 0.81–2.04) and 1.49 (95% CI = 0.77–2.11), respectively (*P* for trend = .05).

Table 1. Age-standardized prevalence of ovarian cancer risk factors according to perineal talc use in 1982*

	Ever perineal talc use, %† (n = 31 789)	No perineal talc use, % (n = 46 841)
Parity		
0	6.3	6.4
1–2	35.0	35.2
⩾3	58.7	58.4
Oral contraceptive use		
Current	0.5	0.6
Past	49.2	49.8
Never	50.4	49.6
Hormone use, postmenopausal women only		
Current	12.1	12.9
Past	20.5	20.4
Never	67.4	66.7
Tubal ligation, yes	17.6	17.6
Cigarette smoking		
Never	44.9	43.2
Past	30.3	28.3
Current	24.9	28.5
Body mass index quintiles, kg/m^2		
<21.0	16.0	22.1
21.0–22.9	20.9	25.4
23.0–24.9	20.1	20.6
25.0–28.9	22.8	19.6
⩾29	19.8	12.0

*Numbers do not always add up to 100% because of missing data or rounding.
†Ever talc use coded as either talc use on perineal area or talc use on sanitary napkins.

Table 2. Talc use and ovarian cancer: 1982 through 1996 (all subtypes included)*

	No. of cases	Person-years	Age-adjusted RR (95% CI)	Multivariate RR† (95% CI)
Talc use on perineum				
Never	186	608 020	1.0 (referent)	1.0 (referent)
<1/wk	43	128 923	1.10 (0.79–1.53)	1.14 (0.81–1.59)
1–6/wk	30	105 186	0.95 (0.65–1.40)	0.99 (0.67–1.46)
Daily	48	142 083	1.09 (0.79–1.49)	1.12 (0.82–1.55)
Talc use on sanitary napkins				
No	242	781 421	1.0 (referent)	1.0 (referent)
Yes	32	111 399	0.89 (0.62–1.29)	0.89 (0.61–1.28)
Ever perineal talc use				
No	179	586 758	1.0 (referent)	1.0 (referent)
Yes	128	397 454	1.05 (0.84–1.32)	1.09 (0.86–1.37)
Talc use, perineal and sanitary napkins				
None	179	586 758	1.0 (referent)	1.0 (referent)
Either talc use on perineum or use on sanitary napkins	103	307 317	1.11 (0.87–1.41)	1.15 (0.90–1.46)
Use on both sanitary napkins and perineum	25	90 137	0.89 (0.58–1.35)	0.90 (0.59–1.37)

*RR = relative risk; CI = confidence interval.
†Multivariate analyses control for age (years), parity (0, 1–2, 3–4, or ⩾5), duration of oral contraceptive use (never or <3 y, 3–5 y, or >5 y), body mass index (body weight in kilograms/height in meters squared: <21, 21.0–22.9, 23.0–24.9, 25.0–28.9, or ⩾29 kg/m^2), tubal ligation history (yes or no), smoking status (never, past, or current), and postmenopausal hormone use (never, past, or current).

Table 3. Talc use and ovarian cancer: 1982–1996 (by histologic subtype)*

Histologic subtype	No. of cases	Person-years	Age-adjusted RR (95% CI)	Multivariate RR† (95% CI)
All serous cancers, ever perineal talc use				
No	101	586 771	1.0 (referent)	1.0 (referent)
Yes	84	397 459	1.23 (0.92–1.64)	1.26 (0.94–1.69)‡
Serous invasive cancers, ever perineal talc use				
No	84	586 771	1.0 (referent)	1.0 (referent)
Yes	76	397 459	1.33 (0.98–1.82)	1.40 (1.02–1.91)‡
Endometrioid cancers, ever perineal talc use				
No	26	586 771	1.0 (referent)	1.0 (referent)
Yes	16	397 459	0.91 (0.49–1.69)	0.91 (0.49–1.87)
Mucinous cancers, ever perineal talc use				
No	30	586 771	1.0 (referent)	1.0 (referent)
Yes	20	397 459	0.98 (0.56–1.73)	0.93 (0.53–1.66)

*RR = relative risk; CI = confidence interval.

†Multivariate analyses controlling for age (years), parity (0, 1–2, or ⩾3), oral contraceptive use (never or ever), and tubal ligation history (yes or no).

‡Multivariate analyses control for age (years), parity (0, 1–2, 3–4, or ⩾5), duration of oral contraceptive use (never or <3 y, 3–5 y, or >5 y), body mass index (body weight in kilograms/height in meters squared: <21, 21.0–22.9, 23.0–24.9, 25.0–28.9, or ⩾29 kg/m^2), tubal ligation history (yes or no), smoking status (never, past, or current), and postmenopausal hormone use (never, past, or current).

Because the talc hypothesis depends on the ability of fibers to migrate up a patent genital tract to the ovaries, we evaluated the risk among women who had reported a tubal ligation and those who had not. Women who were ever talc users and had never had a tubal ligation were not at increased risk of epithelial ovarian cancer compared with women who had not used talc (RR = 0.97; 95% CI = 0.71–1.32). There was no evidence of heterogeneity of RRs between women who had a tubal ligation and women who did not. In addition, when women who had had a tubal ligation or simple hysterectomy were excluded from the analysis, the RR for ever talc use was 1.15 (95% CI = 0.89–1.49). For serous invasive cancers, the RR for women who had never had a tubal ligation was similar to that for women without a tubal ligation; however, the number of case patients who had had a tubal ligation was small (data not shown).

Cosmetic talc may have been more likely to contain asbestos fibers prior to 1976, before voluntary guidelines were proposed *(9)*. As a proxy for early talc use, we assessed risk among women 45 years old or older in 1982. There was no evidence that older women in 1982 were at greater risk of ovarian cancer overall; the RR for ever talc use compared with never talc use for women under 45 years was 0.95 (95% CI = 0.59–1.53) and among women 45 years old or older was 1.13 (95% CI = 0.86–1.47). However, women 45 years old or older in 1982 who ever used talc had a higher risk of serous invasive cancer (RR = 1.51; 95% CI = 1.07–2.15). There was no evidence of effect modification by oral contraceptive use, body mass index, or cigarette smoking for epithelial cancers overall.

Discussion

To our knowledge, this is the first prospective analysis of talc use and ovarian cancer, and it addresses some of the potential limitations of previous case–control studies. Because we ascertained talc exposure prior to case diagnosis, the possibility for recall bias, which has been raised as a potential explanation for previous positive findings in case–control studies *(1)*, is eliminated, and selection bias is reduced. We controlled for known or suspected ovarian cancer risk factors in the analysis, such as parity, oral contraceptive use, tubal ligation history, and body mass index, reducing the potential for uncontrolled confounding.

However, there are several important limitations to our study. The questions on talcum powder use referred to ever use, and we cannot determine the age at which women began using talc or the duration of use. Thus, we were unable to assess the potential effect of talc use before first pregnancy, which has been shown to be a stronger risk factor for ovarian cancer than use after pregnancy in one study *(13)*. The number of lifetime applications of talc has also been associated with increased risk of ovarian cancer in some previous studies *(9,13)*. Our relatively short follow-up period may be inadequate to detect an association if the latency for development of ovarian cancer is more than 15 years. Although we controlled for tubal ligation history, the tubal ligation question was asked as part of a question on contraceptive use; therefore, postmenopausal women and some premenopausal women who were not sexually active may not have responded to the question. Substantial residual confounding is unlikely, since there was no overall association between talc use and tubal ligation in this study. In addition, we excluded women who were postmenopausal in 1976 from analyses stratified by tubal ligation history. Finally, the prevalence of talc use in our study is somewhat higher than that in other studies and may reflect the fact that we asked about frequency of ever use rather than current regular use; this may have contributed to an attenuation of risk due to misclassification of exposure.

The potential effect of talc on the ovaries depends on migration of talc fibers through a patent genital tract, and we would, therefore, expect a stronger association among women without a tubal ligation who had used talc. However, no effect modification was seen by history of tubal ligation. Because we did not have the date of tubal ligation, some women may have begun talc use only after tubal ligation, potentially resulting in misclassification of talc use and attenuation of the RRs.

Since the first study showing an almost twofold increase in risk of ovarian cancer with any perineal talc use *(4)*, most case–control studies have demonstrated positive associations with talc use *(4–13)*, although not all have been statistically significant *(5,6,8)*. Several studies *(9,17–20)* found no overall association between any genital talc use and ovarian cancer. We did not observe a dose–response relationship with talc use, and previous studies also have been inconsistent in this regard. Some studies *(9,13,17)* have demonstrated statistically insignificant trends in risk with increased frequency of talc use, duration of use, and measures of "total lifetime applications," while other studies *(6,8)* have not observed a statistically significant dose response.

With regard to histologic subtypes, a recent study by Cramer et al. *(13)* observed the greatest risk for talc use and invasive serous cancer; however, other

studies found increased risks for endometrioid cancers *(9,12)*, serous cancers *(7)*, and invasive cancers of all subtypes *(12)*. Since serous cancers, which account for more than half of all invasive ovarian cancers, most resemble mesotheliomas, it could be hypothesized that this subtype may be most likely associated with talc use. In our stratification by subtype, we did observe a modest positive association with serous invasive cancers and ever talc use as well as a borderline significant trend for increasing frequency of ever use.

The biologic evidence for the association of talc and ovarian cancer is incomplete. Asbestos has been linked to ovarian cancer in occupational settings and is associated with peritoneal tumors similar to ovarian cancer *(2,3,21)*. Because of the chemical similarity of talc and asbestos, talc also has been implicated as a possible ovarian carcinogen. Talc is able to migrate through the genital tract and gain access to the ovaries because talc fibers have been detected in benign and malignant ovarian tissue *(22)*, although no relation between reported levels of talc exposure and ovarian talc counts has been observed *(23)*. There have been few studies *(24,25)* of talc exposure in animals, and these studies have not demonstrated an increase in ovarian cancer among animals subjected to chronic talc exposure. These data should be interpreted cautiously because there are important anatomic and physiologic differences between rodents and humans, and talc in animals is often administered at high dose via aerosol exposure *(24)*.

In summary, we did not observe an overall association between epithelial ovarian cancer and ever use of talc, and there was no apparent dose response, although we lacked information on duration of talc use. In analyses stratified by histologic subtype, we observed a modest positive association between invasive serous cancer and ever talc use. Our results provide little support for any substantial association between perineal talc use and ovarian cancer risk overall; however, perineal talc use may modestly increase the risk of invasive serous ovarian cancers.

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NOTES

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Exhibit 52

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Talc use, variants of the GSTM1, GSTT1, and NAT2 genes, and risk of epithelial ovarian cancer

Margaret A. Gates[1,2], **Shelley S. Tworoger**[1,2], **Kathryn L. Terry**[1,2,5], **Linda Titus-Ernstoff**[3], **Bernard Rosner**[1,4], **Immaculata De Vivo**[1,2], **Daniel W. Cramer**[2,5], and **Susan E. Hankinson**[1,2]

1*Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

2*Department of Epidemiology, Harvard School of Public Health, Boston, MA*

3*Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH*

4*Department of Biostatistics, Harvard School of Public Health, Boston, MA*

5*Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital, Boston, MA*

Abstract

Epidemiologic evidence suggests a possible association between genital use of talcum powder and risk of epithelial ovarian cancer; however, the biologic basis for this association is not clear. We analyzed interactions between talc use and genes in detoxification pathways (*GSTM1*, *GSTT1* and *NAT2*) to assess whether the talc/ovarian cancer association is modified by variants of genes potentially involved in the response to talc. Our analysis included 1,175 cases and 1,202 controls from a New England-based case-control study and 210 cases and 600 controls from the prospective Nurses' Health Study. We genotyped participants for the *GSTM1* and *GSTT1* gene deletions and three *NAT2* polymorphisms. We used logistic regression to analyze the main effect of talc use, genotype, and gene-talc interactions in each population, and we pooled the estimates using a random effects model. Regular talc use was associated with increased ovarian cancer risk in the combined study population (relative risk=1.36, 95% CI=1.14-1.63; p-trend<0.001). Independent of talc, the genes examined were not clearly associated with risk. However, the talc/ovarian cancer association varied by *GSTT1* genotype and combined *GSTM1/GSTT1* genotype. In the pooled analysis, the association with talc was stronger among women with the *GSTT1*-null genotype (p-interaction=0.03), particularly in combination with the *GSTM1*-present genotype (p-interaction=0.03). There was no clear evidence of an interaction with *GSTM1* alone or *NAT2*. These results suggest that women with certain genetic variants may have a higher risk of ovarian cancer associated with genital talc use. Additional research is needed on these interactions and the underlying biologic mechanisms.

Keywords

Talc; GSTM1; NAT2; ovarian cancer; gene-environment interactions

Correspondence to: Margaret A. Gates.

Correspondence to: Margaret A. Gates, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115; Phone: 617-525-2038; Fax: 617-525-2008; Email: nhmag@channing.harvard.edu.

INTRODUCTION

Genital use of talcum powder has been extensively investigated as a potential risk factor for ovarian cancer. A meta-analysis of 16 previous studies reported an approximate 30% increase in risk of total epithelial ovarian cancer with regular genital exposure to talc (1), and several studies have suggested a stronger association with the serous or serous invasive histologic subtype (2-6). Although the epidemiologic evidence supports a modest association between genital talc use and ovarian cancer risk, the association remains controversial due to the lack of a clear dose-response with increasing frequency or duration of talc use, the possibility of confounding or other biases, and the uncertain biologic mechanism.

No prior studies have assessed gene-talc interactions in ovarian cancer risk, possibly because little is known about which genes may be involved in the biologic response to talc. However, variants of the *glutathione S-transferase M1* (*GSTM1*) and *N-acetyltransferase 2* (*NAT2*) genes appear to modify the association between exposure to asbestos, a known carcinogen that is chemically similar to talc, and risk of malignant mesothelioma (7-10). Talc and asbestos are found together in nature, and prior to 1976 talcum powder was commonly contaminated with asbestos (9). Although this contamination may have contributed to the risk of ovarian cancer associated with talc use, there is also evidence that talc itself may contribute to carcinogenesis, independent of any contamination with asbestos in the past. Talc can induce granulomas and other inflammatory responses *in vivo* (9), and a recent study found that exposing human ovarian stromal and epithelial cells to talc resulted in increased cell proliferation and neoplastic transformation of cells (11). Talc also appears to increase cellular production of reactive oxygen species (11). Interestingly, serous ovarian cancers morphologically resemble peritoneal malignant mesotheliomas (12), suggesting a possible rationale for the stronger association between talc and risk of serous or serous invasive cancers observed in some studies.

Based on similarities between talc and asbestos and the evidence for gene-asbestos interactions in malignant mesothelioma, we examined whether the association between genital talc exposure and ovarian cancer risk is modified by variants of the *NAT2* and *GSTM1* genes, as well as the related *glutathione S-transferase T1* (*GSTT1*) gene. The *GSTM1* and *GSTT1* genes produce enzymes involved in the metabolism of carcinogens and reactive oxygen species (13). These genes are homozygously deleted in approximately 50% (*GSTM1*) and 20% (*GSTT1*) of Caucasians, resulting in complete loss of enzymatic activity (14,15). The NAT2 enzyme catalyzes the deactivation of xenobiotics via *N*-acetylation, but can also activate certain substrates via *O*-acetylation (16). Individuals with two *NAT2* slow acetylator alleles, approximately 60% of individuals in Caucasian populations, have decreased rates of *N*- and *O*-acetylation (17-20). We hypothesized that the association between talc use and ovarian cancer risk would be stronger among individuals with the *GSTM1* null, *GSTT1* null, and *NAT2* slow acetylator genotypes, due to decreased metabolism of free radicals and other products of the biologic response to talc. We examined these gene-talc interactions, as well as the main effect of talc use and each genotype, in two study populations with a total of 1,385 ovarian cancer cases.

METHODS

New England Case-Control Study

The New England Case-Control Study (NECC) consists of 1,231 epithelial ovarian cancer cases and 1,244 controls from Massachusetts and New Hampshire. Participants were enrolled in the study in two phases, from May 1992 to March 1997 (phase 1; 563 cases and 523 controls) or from July 1998 to July 2003 (phase 2; 668 cases and 721 controls). Participants completed a detailed questionnaire on potential risk factors for ovarian cancer and covariates of interest during an in-person interview with a trained interviewer. To avoid capturing changes related

to disease status, interviewers asked participants about exposures that occurred at least one year prior to the date of diagnosis for cases or the interview date for controls. The institutional review boards of Brigham and Women's Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent.

During the two study phases, NECC researchers identified 2,347 incident cases of ovarian cancer through hospital tumor boards and state cancer registries; 1,845 (79%) of these cases were eligible, and 71% of the eligible cases were enrolled in the study. Study investigators identified potential controls using random digit dialing, drivers' license records, and Massachusetts town resident lists. Controls were frequency-matched to cases by age and state of residence. Of the potentially eligible controls contacted by investigators during phase 1, 68% were eligible and agreed to participate. During phase 2, 197 potential controls declined to be contacted by returning a postcard to “opt out” of the study; of the remaining potentially eligible controls who were contacted, 67% were eligible and enrolled in the study. The eligibility criteria and the reasons for non-enrollment of eligible cases are described elsewhere (21).

Over 95% of study participants provided a blood specimen at study enrollment. NECC researchers separated the heparinized blood samples into plasma, red blood cell, and buffy coat (white blood cell) components, extracted DNA from the buffy coat using Qiagen DNA extraction (Qiagen Inc., Valencia, CA), and stored the extracted DNA in freezers at a temperature of -80°C.

Nurses' Health Study

In 1976, 121,701 female registered nurses between the ages of 30 and 55 responded to a mailed questionnaire about known and suspected risk factors for disease, leading to the establishment of the Nurses' Health Study (NHS). Study participants completed follow-up questionnaires every two years, providing information on new diagnoses of disease and updated information on risk factors. Participation in the study has remained high throughout follow-up; between 1976 and 2004 the percentage of follow-up information obtained (questionnaire responses plus deaths) was 95.3%. The corresponding follow-up percentages for women who provided a white blood cell or cheek cell specimen were 98% and 99%, respectively. The Institutional Review Board of Brigham and Women's Hospital, Boston, MA approved both the NHS and this analysis, and all participants provided implied consent by completing and returning the baseline questionnaire.

In 1989 and 1990, 32,826 participants submitted a blood sample for use in genetic and other biomarker analyses. Details of the blood collection are described elsewhere (22). Between 2001 and 2004, 33,040 women without a blood specimen provided a buccal cell specimen. We used a mouthwash protocol to collect the buccal cell samples, based on evidence that this method provides slightly higher DNA yield and quality, compared with collection using a cytobrush (23). We extracted DNA from each specimen within one week of receipt using Qiagen DNA extraction (Qiagen Inc., Valencia, CA), and stored the DNA at -80°C.

NHS nested case-control study

We collected information on new diagnoses of ovarian cancer on each questionnaire, and we also obtained information on deaths due to ovarian cancer through family members, the National Death Index, and the U.S. Postal Service. We confirmed each diagnosis using methods described previously (24). For this analysis, we included all cases with a DNA specimen available from prior to diagnosis (incident cases), as well as cases who submitted a DNA specimen within four years after diagnosis (prevalent cases). We included the prevalent cases in the analysis due to the similarity of characteristics of these cases and the incident cases, and

also because the interval of four years between diagnosis and DNA collection was less than the average survival time of 65.7 months for the incident cases. All cases were diagnosed prior to June 1, 2004 and had no history of a prior cancer, other than non-melanoma skin cancer.

We randomly selected three controls per case from the study participants who gave a buccal cell or blood specimen, who had not had a bilateral oophorectomy prior to the date of diagnosis of the matched case, and who had no history of cancer, other than non-melanoma skin cancer, as of the cycle of diagnosis of the case. We excluded 30 controls from the analysis due to unavailability of genotyping data (n=28) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n=2). Cases and controls were matched on month and year of birth, DNA type, and menopausal status at diagnosis. For the blood collection, cases and controls were additionally matched on menopausal status and postmenopausal hormone (PMH) use status at blood draw, month/year and time of day of blood draw, and fasting status at blood draw, since these control selections were also used for analyses of plasma hormones and other biomarkers (25).

Exposure assessment

The phase 1 and 2 NECC questionnaires included multiple questions about regular use of talcum, baby or deodorizing powder as an adult. Specific questions asked about type of use (as a dusting powder to the genital area, sanitary napkins, underwear, or non-genital areas), frequency of use, age at first use, number of years used, and brand of powder used. The 1982 NHS questionnaire requested information on whether the participant had ever commonly applied talcum, baby, or deodorizing powder to the perineal area (no, <once/week, 1-6 times/week, or daily) or to sanitary napkins (yes/no). For this analysis, we defined regular genital talc use as application of powder to the genital/perineal region at least once per week. We also created a categorical variable for frequency of talc use, using the categories from the NHS questionnaire.

Genotyping methods

Genotyping was performed at the Dana Farber/Harvard Cancer Center High Throughput Genotyping Core (for the *NAT2* polymorphisms and NHS *GSTM1* and *GSTT1* gene deletions) and the Molecular Epidemiology Research Laboratory at the Harvard School of Public Health (for the NECC *GSTM1* and *GSTT1* gene deletions). All samples were genotyped for three single nucleotide polymorphisms that identify the *NAT2*5*, *NAT2*6*, and *NAT2*7* alleles. These alleles account for over 99% of slow acetylator alleles in Caucasian populations (16,26). The *NAT2* I114T (rs1801280), R197Q (rs1799930), and G286E (rs1799931) polymorphisms were genotyped using the 5' nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. Individuals with two slow acetylator alleles were classified as *NAT2* slow acetylators, while individuals with zero or one slow acetylator allele were classified as rapid acetylators.

The NECC samples were genotyped for the *GSTM1* and *GSTT1* gene deletions using multiplex polymerase chain reaction (PCR), and the PCR products were resolved on a 1.5% agarose gel. The NHS samples were genotyped for the two gene deletions using Taqman realtime PCR in 384-well format. For both the multiplex and real-time PCR assays, individuals were considered to have the *GSTM1* or *GSTT1* null genotype if no PCR product was present for the respective gene; all other individuals were classified as *GSTM1* or *GSTT1* present.

All DNA samples were whole genome amplified prior to genotyping. Laboratory personnel blinded to the case-control status of the samples performed all genotyping, and each plate included blinded replicate samples for quality control purposes. The replicate samples were

100% concordant for all genotypes except the NECC *GSTM1* and *GSTT1* gene deletions, which were 98% and 95% concordant respectively.

Statistical analysis

We used a chi-square test to examine whether the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium in each population, and also to examine the distribution of each genotype by case-control status. We conducted all analyses separately in the NHS and NECC populations using consistent exposure and covariate definitions and, after testing for heterogeneity in the results, pooled the estimates using a random effects model (27). We used conditional (NHS) and unconditional (NECC and NHS) logistic regression to model the multivariable-adjusted odds ratio (as an estimate of the relative risk [RR]) and 95% confidence interval (CI) for the main effect of genital talc use, the main effect of each gene, and each combined gene-talc variable. We tested for a linear trend with increasing frequency of talc use by using a continuous variable weighted by the midpoint of each frequency category, and we calculated the *p*-value for trend using the Wald test. To assess effect modification by genotype, we used unconditional logistic regression to model the association between talc use and ovarian cancer risk within each genotype stratum, and we calculated the *p*-value for interaction using the chi-square test for the difference between the log likelihoods for models with and without interaction terms between regular genital talc use and genotype. In addition to the analyses of total ovarian cancer, we examined associations with the serous invasive histologic subtype, based on evidence from prior studies that risk of this subtype may be more strongly associated with talc use.

We adjusted all analyses for the matching factors, duration of oral contraceptive use, parity, tubal ligation, body mass index (BMI), and duration of PMH use. Women with missing data for the continuous covariates were assigned the median value of the covariate for their study population. In the NHS, where covariate data are available from multiple questionnaire cycles, we used the data from two cycles (two to four years) prior to the cycle of diagnosis for each case and their matched controls, for consistency with the timeframe of the NECC covariate data. We examined additional covariates as potential confounders, including physical activity, smoking history, menopausal status, age at menopause, breastfeeding duration, and family history of ovarian or breast cancer, but did not include them in the final model because they did not substantially change our estimates. We performed all analyses using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Our study population included 1,175 cases and 1,202 frequency-matched controls from the NECC and 210 cases and 600 matched controls from the NHS, for a total of 1,385 ovarian cancer cases and 1,802 controls. Of the NHS cases, 49 were prevalent and 161 were incident with respect to the time of DNA collection. Characteristics of the NHS prevalent and incident cases were generally similar, although a higher percentage of the prevalent cancers were endometrioid (20% vs. 9%) and a lower percentage were invasive (76% vs. 86%). In the NECC, 618 cases had serous histology (53%), 450 were serous invasive (38%), 153 were mucinous (13%), 172 were endometrioid (15%), and 232 had other/undifferentiated histology (20%). In the NHS, 111 cases were serous (53%), 93 were serous invasive (44%), 23 were mucinous (11%), 25 were endometrioid (12%), and 51 had other/poorly differentiated histology (24%).

Over 96% of the NECC participants and 98% of the NHS participants were of self-reported European ancestry. In analyses restricted to these participants, the results were similar to those for the entire study population; we therefore included all participants in our analyses to maximize our sample size. The distributions of ovarian cancer risk factors were similar in the NECC and NHS populations, although on average the NHS participants were older, had higher

parity, and were more likely to have used PMH, in part due to differences in the NECC and NHS age distributions (Table 1). Within each study population the cases and controls differed with respect to the known risk factors for ovarian cancer. In addition, in the NECC the cases had higher mean BMI than the controls, and a larger percentage of the cases reported a history of genital talc use. The NHS prevalent and incident cases had similar BMI, tubal ligation history, duration of PMH use, duration of lactation, and genital talc use history; however, the prevalent cases were, on average, slightly younger (60 vs. 62 years), less likely to be postmenopausal (71% vs. 87%), and had lower parity (2.7 vs. 3.1 children), later age at menarche (13.1 vs. 12.5 years), and a longer mean duration of oral contraceptive use (60 vs. 41 months; results not shown).

In the NECC, women with a history of regular genital talc use were older, had higher mean BMI, were less likely to have ever used oral contraceptives, were more likely to be postmenopausal, and were more likely to have used PMH (Table 2). Among parous women in the NHS, the mean age at first birth was lower for regular talc users. In addition, NHS participants who regularly used talc were less likely to have a history of smoking or tubal ligation. There was no difference in the genotype frequencies by genital talc use history in either study population.

All *p*-values for the tests for heterogeneity comparing the NECC and NHS results were greater than 0.05. Talc use was associated with increased risk of ovarian cancer in both study populations, although the confidence intervals were wide in the NHS due to the limited sample size (Table 3). In the pooled analysis, the relative risk for the association with regular genital talc use was 1.36 (95% CI=1.14-1.63) for total ovarian cancer and 1.60 (95% CI=1.26-2.02) for the serous invasive subtype. In addition, there were highly significant trends between increasing frequency of talc use and risk of both total and serous invasive ovarian cancer in the NECC (*p*-trend=0.002 for total and <0.001 for serous invasive ovarian cancer) and pooled analyses (*p*-trend<0.001 for both total and serous invasive ovarian cancer). Regular genital talc use was not significantly associated with risk of the endometrioid (RR=1.41, 95% CI=0.97-2.05) or mucinous (RR=1.28, 95% CI=0.85-1.92) histologic subtypes in the pooled analysis. In the NECC, use of talcum powder on non-genital body areas was unassociated with ovarian cancer risk (multivariable-adjusted RR, also adjusted for genital talc use=0.91, 95% CI=0.73-1.12).

Among the controls in each population, the genotype frequencies for the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium and the distributions of the *GSTM1* null, *GSTT1* null, and *NAT2* slow acetylator genotypes were consistent with previous reports of Caucasian populations (19,28,29). Comparing the prevalent and incident cases in the NHS, a nonsignificantly higher percentage of the prevalent cases were *NAT2* slow acetylators (67% vs. 56%), but the *GSTM1* and *GSTT1* genotype distributions did not differ for the prevalent and incident cases (results not shown).

None of the genotypes examined were associated with ovarian cancer risk in the NECC or pooled analyses (Table 4). In the NHS, individuals with the *NAT2* slow acetylator genotype had a significant 35% decrease in ovarian cancer risk (RR=0.65, 95% CI=0.45-0.95). The combined *GSTM1* null/*NAT2* slow acetylator and *GSTT1* null/*NAT2* slow acetylator genotypes were also inversely associated with risk in the NHS (RR=0.57, 95% CI=0.33-0.98 and RR=0.51, 95% CI=0.26-0.99, respectively), when compared with the *GSTM1* or *GSTT1* present, *NAT2* rapid acetylator genotype. However, these associations were no longer statistically significant when pooled with the NECC estimates.

In analyses stratified by genotype, the association between regular genital talc use and risk of total ovarian cancer was stronger among women with the *GSTT1* null and combined *GSTM1*

present/*GSTT1* null genotypes (Table 5). In the pooled analysis, the relative risk for the association with regular genital talc use was 2.1 (95% CI=1.4-3.2) for women with the *GSTT1* null genotype (*p*-interaction=0.03) and 2.8 (95% CI=1.6-5.0) for women with the *GSTM1* present/*GSTT1* null genotype (*p*-interaction=0.03). The association with the serous invasive subtype was also stronger within these genotype strata, although the *p*-values for interaction were not statistically significant. The pooled relative risk was 2.4 (95% CI=1.4-4.0) for the *GSTT1* null stratum and 4.8 (95% CI=2.1-11) for the combined *GSTM1* present/*GSTT1* null stratum. The results were consistent in both study populations (results not shown), although the *p*-values for interaction were statistically significant only in the pooled analysis. There was also evidence of a stronger association between regular talc use and risk of serous invasive cancer among women with the *GSTM1* present genotype, but this interaction was not statistically significant.

We additionally analyzed the association between combined gene-talc variables, compared to a common referent group (wild-type genotype and no talc use), and risk of total and serous invasive ovarian cancer. The results of these analyses were similar to the stratified results presented in table 5, and are therefore included only as a supplementary table. We also examined interactions between regular genital talc use and combined *GSTM1/NAT2* and *GSTT1/NAT2* genotype (results not shown). The *GSTT1* null/*NAT2* slow acetylator genotype seemed to increase the risk of total and serous invasive ovarian cancer associated with talc use. However, these analyses were based on small numbers, especially for certain combinations of the genotype and talc variables, and none of the *p*-values for interaction were significant.

In analyses restricted to the NHS incident cases or the NHS cases and controls with a blood specimen, the results were similar to those for the total NHS study population (results not shown).

DISCUSSION

These results provide additional support for a main effect of genital talc exposure on risk of epithelial ovarian cancer. The presence of a significant trend between frequency of talc use and risk of total and serous invasive ovarian cancer in the NECC and pooled analyses further strengthens the evidence for an association, as most previous studies have not observed a dose-response with increasing frequency or duration of talc use (1,5). The results of our gene-environment analyses suggest that genes in detoxification pathways may be involved in the biologic response to talc, and that the association between genital talc use and risk of ovarian cancer may vary by genotype. In particular, women with the *GSTT1* null genotype and the combined *GSTM1* present/*GSTT1* null genotype had a stronger association between talc use and ovarian cancer risk. The evidence for these interactions was consistent in two independent study populations, and the *p*-values for interaction were statistically significant in a pooled analysis of the two populations. However, the direction of the interaction with combined *GSTM1/GSTT1* genotype was unexpected based on the known function of these genes.

Although prior analyses of the talc/ovarian cancer association in the NHS and the NECC have been published, our study includes an additional 612 NECC cases and 679 NECC controls and eight additional years of follow-up in the NHS (3,4). In the previous analysis of the NECC, Cramer et al. observed a significant positive association between talc use and risk of both total and serous invasive ovarian cancer. In addition, there was a significant trend with lifetime number of talc applications, after excluding applications during non-ovulatory intervals (*p*-trend=0.02), but no trend with duration or frequency of talc use (3). In the only prospective study of this association, Gertig et al. reported a significant association between talc use and risk of the serous invasive subtype in the NHS, but no association with risk of total ovarian cancer (4). Our findings are consistent with the previous reports for these study populations,

although our analysis differs from the prior studies in that we defined our primary exposure variable as genital use of talc at least once per week, based on the assumption that habitual talc use is more likely to be recalled accurately and more likely to be associated with ovarian cancer risk. Our findings are also consistent with meta-analyses of this association (1,30).

The controversy regarding the existence of an association between talc and ovarian cancer has stemmed in part from the lack of a clear mechanism for the association. Although talc and asbestos are chemically similar, their biologic effects may differ, since talc does not appear to be a lung carcinogen (31). In addition, it is unclear whether talc applied to the perineum can reach the ovaries, although some studies have demonstrated that inert particles can travel through the female genital tract to the fallopian tubes and ovaries (32,33), and others have found talc particles in ovarian tissue (34-37). Recent studies have suggested additional potential mechanisms for an association between talc and ovarian cancer. Talc particles can induce an inflammatory response *in vivo*, which may be important in ovarian cancer risk (38). Normal ovarian cells treated with talc are more likely to undergo cell proliferation and neoplastic transformation, and cellular generation of reactive oxygen species increases with increasing exposure to talc (11). Recent studies by Cramer and colleagues also support the possibility of an immune-mediated mechanism for an association between talc and ovarian cancer and suggest that exposure of the lower genital tract to talc may be sufficient to cause changes, such as production of heat shock proteins, accumulation of talc in pelvic lymph nodes, or decreased levels of anti-MUC1 antibodies, that could increase ovarian cancer risk (39-41).

Although no prior studies have examined gene-talc interactions, the indication of a possible immune-related mechanism between talc and ovarian carcinogenesis and the evidence for gene-asbestos interactions suggest that genes involved in detoxification and inflammatory pathways could be important in the response to talc. Previous studies have indicated that *NAT2* and *GSTM1* genotype may modify the association between asbestos exposure and risk of malignant mesothelioma; however, not all studies have been consistent (7,8,42,43), and for *NAT2* the direction of the interaction differed in studies conducted in Finnish and Italian populations (7,8,42,44). This suggests that interactions with these genes may be complex and might depend on additional factors, such as the presence of other gene variants, the type of asbestos, or the level of asbestos exposure (8).

The *GSTM1* and *GSTT1* genes produce enzymes that metabolize products of oxidative stress and catalyze the detoxification of carcinogens and other xenobiotics (45). The *GSTM1* deletion and to a lesser extent the *GSTT1* deletion may increase the risk of certain cancers; however, our study and previous analyses do not support a direct association between the *GSTM1* or *GSTT1* gene deletion and risk of ovarian cancer (13,17,28). While there is some overlap in GST substrate specificity, there are also differences in the substrates metabolized by the GSTM1 and GSTT1 enzymes, which could help to explain the opposite direction of the interactions we observed between talc use and *GSTM1* and *GSTT1* genotype (13,17,45). In studies of pleural malignant mesothelioma, the *GSTM1* null genotype was associated with increased risk (7,8,42,43) while the *GSTT1* null genotype was unassociated with risk of malignant mesothelioma (8,42,43) but was associated with a significant decrease in risk of asbestosis in one study (46), providing support that some functions of the GSTM1 and GSTT1 enzymes may differ. The direction of the associations between the *GSTM1* and *GSTT1* deletions and risk of asbestos-related disease was opposite to the direction of the interactions with talc observed in our study; this could potentially be due to differences in the chemical structures of talc and asbestos or differences in the by-products produced during the biologic response to talc and asbestos. The NAT2 enzyme catalyzes the transfer of an acetyl group to its substrates, including carcinogens such as heterocyclic and aromatic amines, which can result in either activation or deactivation of these substances (17,20). Approximately 60% of Caucasians have two *NAT2* slow acetylator alleles and consequently have decreased rates of acetylation, which

can either increase or decrease the risk of certain cancers depending on the substrate and the cancer site (17,20). To our knowledge, no previous studies have examined the association between the *NAT2* slow acetylator genotype and ovarian cancer risk. We did not observe strong evidence of a main effect of *NAT2* genotype or an interaction between *NAT2* genotype and talc exposure.

The novelty of this analysis and the assessment of gene-talc interactions in two independent study populations, one with a large number of cases and the other with prospective data on talc use and ovarian cancer incidence, are strengths of this study. However, although the pooled analysis included a large number of cases and controls, our power was still insufficient to detect interactions with certain combinations of genes and for specific histologic subtypes. In addition, while both study populations had extensive covariate data, the use of common exposure and covariate definitions resulted in the loss of some detail, particularly for the NECC. Information on talc use was only collected in 1982 in the NHS, so it is possible that some participants were misclassified with respect to their talc use history. However, the number of participants who began using talc after 1982, when the participants were between 36 and 61 years of age, is most likely small. Although we do not have data on age at initiation of talc use in the NHS, in the NECC approximately 95% of controls with a history of regular genital talc use reported first using talc before age 35. Recall or selection bias may have affected the results of the NECC analyses, due to the retrospective study design. However, the consistency of the NECC and NHS results suggests that biases related to study design were not a major problem, since, with the exception of the DNA for a subset of the cases, the NHS data were collected prospectively. In addition, the exposure definition of genital talc use at least once per week may have decreased the influence of recall bias in this analysis, since habitual talc use is likely to be recalled more accurately than sporadic use.

In summary, our findings suggest that variants of the *GSTM1* and *GSTT1* genes may modify the association between genital talc use and risk of total and serous invasive ovarian cancer. However, additional research is needed to confirm these findings and to explore potential mechanisms for these interactions, particularly for the stronger talc/ovarian cancer association among women with the *GSTM1* present/*GSTT1* null genotype. If confirmed, these findings would strengthen the evidence for the carcinogenicity of talc to the ovarian epithelium.

Supplementary Table

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of ovarian cancer cases and controls in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

Characteristic	NECC			NHS[//]		
	Cases	Controls	*P*[**]	Cases	Controls	*P*[**]
N	1175	1202		210	600	
Mean value (standard deviation)						
Age in years[*]	51 (13)	51 (13)	0.37	62 (8)	62 (8)	0.93
Parity among parous women	2.5 (1.3)	2.8 (1.5)	<0.001	3.0 (1.3)	3.3 (1.5)	0.007
Duration oral contraceptive use (months)[†]	52 (54)	61 (55)	0.006	46 (42)	53 (49)	0.22
Body mass index (kg/m^2)	26.3 (6.3)	25.7 (5.5)	0.02	25.7 (5.0)	25.7 (4.5)	0.94
Duration PMH use (months)[†]	78 (86)	74 (71)	0.64	96 (84)	85 (68)	0.18
Duration of lactation (months)[‡]	3.4 (8.6)	5.9 (12.2)	<0.001	2.9 (2.3)	3.6 (2.5)	0.002
Percent of study population						
Parous	68	81	<0.001	89	93	0.05
Ever user of oral contraceptives	48	60	<0.001	42	45	0.57
History of tubal ligation	14	18	0.007	14	21	0.02
Ever user of PMH	17	20	0.14	71	63	0.02
Family history of ovarian cancer	5.1	2.8	0.004	9.1	3.7	0.002
Any history of genital talc use	29	24	0.003	40	39	0.79
Regular genital talc use (>=once/week)	27	20	<0.001	29	24	0.15
Daily genital talc use	16	12	0.006	18	13	0.08
Genotype frequencies, %						
GSTM1 null	51	53	0.42	48	52	0.36
GSTT1 null	21	22	0.85	19	21	0.45
NAT2 slow acetylator[§]	63	64	0.74	59	67	0.05

[*] Cases and controls in each study population were matched (NHS) or frequency-matched (NECC) on age

[†] Duration of oral contraceptive use and postmenopausal hormone (PMH) use among ever users

[‡] Total duration among parous women

[§] *NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E

[//] In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (two to four years) prior to the date of diagnosis for each case was used for the case and their matched controls

[**] *P*-values calculated using proc ttest (continuous variables) or a chi-square test (binary variables)

Table 2

Characteristics of participants in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS) by history of regular genital talc use (at least once per week)

Characteristic	NECC			NHS[§]		
	No Regular Talc Use	Regular Talc Use	*P*[‖]	No Regular Talc Use	Regular Talc Use	*P*[‖]
Mean value (standard deviation)						
Age in years	50 (13)	53 (12)	<0.001	61 (8)	62 (8)	0.64
Parity among parous women	2.7 (1.4)	2.7 (1.4)	0.64	3.2 (1.4)	3.3 (1.5)	0.40
Age at first birth among parous women	25.0 (5.1)	24.6 (4.9)	0.22	25.0 (3.5)	24.4 (3.0)	0.03
Duration oral contraceptive use (months)[*]	58 (55)	54 (54)	0.24	53 (49)	43 (42)	0.08
Body mass index (kg/m^2)	25.7 (5.7)	27.0 (6.4)	<0.001	25.6 (4.6)	26.2 (4.8)	0.13
Duration PMH use (months)[*]	75 (74)	78 (86)	0.68	90 (74)	83 (70)	0.38
Duration of lactation (months)[†]	4.8 (10.8)	4.3 (10.3)	0.38	3.5 (2.5)	3.2 (2.4)	0.20
Physical activity (hours/week)	2.8 (5.0)	2.4 (3.8)	0.06	3.0 (2.3)	3.1 (2.4)	0.61
Percent of study population						
Parous	74	75	0.75	93	92	0.81
Ever user of oral contraceptives	55	50	0.03	44	44	0.96
History of tubal ligation	16	16	0.97	22	13	0.008
Postmenopause	45	54	<0.001	81	81	0.86
Ever user of PMH	17	26	<0.001	66	64	0.73
Ever smoker	53	55	0.35	57	47	0.02
Family history of ovarian cancer	3.9	4.1	0.82	4.5	6.4	0.31
Genotype frequencies, %						
GSTM1 null	52	52	0.72	51	49	0.66
GSTT1 null	21	22	0.59	22	17	0.15
NAT2 slow acetylator[‡]	63	66	0.23	65	63	0.58

[*] Duration of oral contraceptive use and postmenopausal hormone (PMH) use among ever users

[†] Total duration among parous women

[‡] *NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E

[§] In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (two to four years) prior to the date of diagnosis for each case was used for the case and their matched controls

[‖] *P*-values calculated using proc ttest (continuous variables) or a chi-square test (binary variables)

Table 3

Relative risks (RRs) and 95% confidence intervals (CIs) for the association between genital talc use and ovarian cancer risk in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

	NECC[†]			NHS[†]			Pooled[‡]
	Cases (%)	Ctrls (%)	RR (95% CI)	Cases (%)	Ctrls (%)	RR (95% CI)	RR (95% CI)
Total epithelial ovarian cancer:							
N[*]	1175	1202		210	600		
Regular genital talc use (>=once/week)							
No	859 (73.2)	957 (79.7)	1.00 (ref.)	138 (70.8)	414 (76.0)	1.00 (ref.)	1.00 (ref.)
Yes	314 (26.8)	244 (20.3)	1.40 (1.15, 1.70)	57 (29.2)	131 (24.0)	1.24 (0.83, 1.83)	1.36 (1.14, 1.63)
Frequency of genital talc use							
Never	832 (70.9)	916 (76.3)	1.00 (ref.)	120 (61.5)	352 (64.6)	1.00 (ref.)	1.00 (ref.)
<once/week	27 (2.3)	41 (3.4)	0.72 (0.43, 1.19)	18 (9.2)	62 (11.4)	0.98 (0.54, 1.79)	0.82 (0.55, 1.20)
1-6 times/week	123 (10.5)	96 (8.0)	1.33 (1.00, 1.79)	22 (11.3)	61 (11.2)	1.01 (0.57, 1.79)	1.26 (0.97, 1.63)
Daily	191 (16.3)	148 (12.3)	1.41 (1.10, 1.79)	35 (18.0)	70 (12.8)	1.44 (0.88, 2.37)	1.41 (1.14, 1.76)
P-trend[§]			0.002			0.18	<0.001
Serous invasive ovarian cancer:							
N[*]	450	1202		93	263		
Regular genital talc use (>=once/week)							
No	310 (69.0)	957 (79.7)	1.00 (ref.)	60 (68.2)	177 (73.8)	1.00 (ref.)	1.00 (ref.)
Yes	139 (31.0)	244 (20.3)	1.62 (1.26, 2.09)	28 (31.8)	63 (26.3)	1.48 (0.82, 2.68)	1.60 (1.26, 2.02)
Frequency of genital talc use							
Never	299 (66.6)	916 (76.3)	1.00 (ref.)	54 (61.4)	151 (62.9)	1.00 (ref.)	1.00 (ref.)
<once/week	11 (2.4)	41 (3.4)	0.65 (0.32, 1.33)	6 (6.8)	26 (10.8)	0.79 (0.29, 2.11)	0.70 (0.39, 1.24)
1-6 times/week	56 (12.5)	96 (8.0)	1.56 (1.08, 2.26)	12 (13.6)	25 (10.4)	1.64 (0.71, 3.79)	1.58 (1.12, 2.21)
Daily	83 (18.5)	148 (12.3)	1.61 (1.18, 2.20)	16 (18.2)	38 (15.8)	1.34 (0.65, 2.76)	1.56 (1.17, 2.08)
P-trend[§]			<0.001			0.29	<0.001

[*] Frequencies do not add up to total N due to missing data on talc use

[†] Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

[‡] *P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.38

[§] Weighted by the midpoint of each category of genital talc use frequency and calculated using the Wald test

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Table 4

Relative risks (RRs) and 95% confidence intervals (CIs) for the association between *GSTM1*, *GSTT1*, and *NAT2* genotype and epithelial ovarian cancer risk in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

	NECC†			NHS†			Pooled‡
	Cases (%)	Ctrls (%)	RR (95% CI)	Cases (%)	Ctrls (%)	RR (95% CI)	RR (95% CI)
N*	1175	1202		210	600		
GSTM1 genotype							
Present	573 (49.1)	567 (47.4)	1.00 (ref.)	102 (52.3)	268 (48.5)	1.00 (ref.)	1.00 (ref.)
Null	594 (50.9)	628 (52.6)	0.93 (0.79, 1.10)	93 (47.7)	285 (51.5)	0.83 (0.58, 1.17)	0.91 (0.78, 1.06)
GSTT1 genotype							
Present	919 (78.8)	938 (78.5)	1.00 (ref.)	157 (81.3)	439 (78.8)	1.00 (ref.)	1.00 (ref.)
Null	247 (21.2)	257 (21.5)	0.98 (0.80, 1.21)	36 (18.7)	118 (21.2)	0.87 (0.57, 1.33)	0.96 (0.80, 1.16)
NAT2 genotype							
Rapid/intermediate acetylator	387 (36.8)	405 (36.1)	1.00 (ref.)	77 (41.0)	182 (33.0)	1.00 (ref.)	1.00 (ref.)
Slow acetylator	665 (63.2)	717 (63.9)	0.97 (0.81, 1.15)	111 (59.0)	369 (67.0)	0.65 (0.45, 0.95)	0.82 (0.57, 1.20)
Combined *GSTM1/GSTT*1 genotype							
Both present	445 (38.2)	430 (36.0)	1.00 (ref.)	81 (44.3)	206 (39.2)	1.00 (ref.)	1.00 (ref.)
M1 null, *T1* present	474 (40.7)	508 (42.5)	0.91 (0.76, 1.10)	68 (37.2)	208 (39.5)	0.82 (0.54, 1.22)	0.89 (0.75, 1.06)
M1 present, *T1* null	128 (11.0)	137 (11.5)	0.94 (0.71, 1.24)	17 (9.3)	49 (9.3)	0.98 (0.52, 1.84)	0.94 (0.73, 1.22)
Both null	119 (10.2)	120 (10.0)	0.94 (0.70, 1.26)	17 (9.3)	63 (12.0)	0.65 (0.34, 1.24)	0.88 (0.67, 1.15)
Combined *GSTM1/NAT2* genotype							
GSTM1 present, *NAT2* rapid	195 (18.6)	188 (16.9)	1.00 (ref.)	37 (21.0)	85 (16.4)	1.00 (ref.)	1.00 (ref.)
GSTM1 null, *NAT2* rapid	189 (18.1)	214 (19.2)	0.82 (0.61, 1.09)	35 (19.9)	91 (17.5)	0.96 (0.53, 1.74)	0.84 (0.65, 1.09)
GSTM1 present, *NAT2* slow	315 (30.1)	343 (30.7)	0.86 (0.66, 1.11)	56 (31.8)	161 (31.0)	0.87 (0.51, 1.50)	0.86 (0.68, 1.09)
GSTM1 null, *NAT2* slow	347 (33.2)	371 (33.2)	0.88 (0.68, 1.14)	48 (27.3)	183 (35.2)	0.57 (0.33, 0.98)	0.76 (0.50, 1.14)
Combined *GSTT1/NAT2* genotype							
GSTT1 present, *NAT2* rapid	296 (28.3)	312 (28.0)	1.00 (ref.)	52 (29.7)	144 (27.5)	1.00 (ref.)	1.00 (ref.)
GSTT1 null, *NAT2* rapid	88 (8.4)	90 (8.1)	1.03 (0.73, 1.45)	17 (9.7)	30 (5.7)	1.55 (0.76, 3.16)	1.11 (0.81, 1.52)
GSTT1 present, *NAT2* slow	519 (49.7)	562 (50.4)	0.97 (0.79, 1.19)	90 (51.4)	270 (51.6)	0.87 (0.57, 1.33)	0.95 (0.79, 1.14)
GSTT1 null, *NAT2* slow	142 (13.6)	152 (13.6)	0.98 (0.74, 1.31)	16 (9.1)	79 (15.1)	0.51 (0.26, 0.99)	0.76 (0.40, 1.43)

*Frequencies do not add up to total N due to missing genotype data

†Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

‡*P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.06

Table 5

Pooled relative risks (RRs) and 95% confidence intervals (CIs) for the association between regular talc use and ovarian cancer risk, stratified by genotype, in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)[*†]

	All cancers		Serous invasive cancers		Cases and controls in pooled analysis					
					All cases		Serous inv.		Controls	
	Regular talc use		Regular talc use		Regular talc		Regular talc		Regular talc	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Gene/stratum										
GSTM1 genotype										
Present (+)	1.0 (ref.)	1.6 (1.2, 2.0)	1.0 (ref.)	2.0 (1.4, 2.8)	480	189	173	90	646	165
Null (-)	1.0 (ref.)	1.3 (1.0, 1.6)	1.0 (ref.)	1.4 (1.0, 1.9)	498	179	190	76	690	198
P-interaction[§]	0.13		0.08							
GSTT1 genotype										
Present (+)	1.0 (ref.)	1.2 (1.0, 1.5)	1.0 (ref.)	1.5 (1.2, 2.0)	785	278	288	129	1035	301
Null (-)	1.0 (ref.)	2.1 (1.4, 3.2)	1.0 (ref.)	2.4 (1.4, 4.0)	194	87	71	38	300	67
P-interaction[§]	0.03		0.18							
NAT2 genotype										
Rapid/intermediate acetylator	1.0 (ref.)	1.5 (1.1, 2.0)	1.0 (ref.)	1.9 (1.2, 2.8)	330	128	123	57	459	113
Slow acetylator	1.0 (ref.)	1.4 (1.1, 1.8)	1.0 (ref.)	1.6 (1.2, 2.1)	552	217	204	96	819	233
P-interaction[§]	0.60		0.58							
GSTM1/GSTT1 genotype[‡]										
GSTM1+, *GSTT1 +*	1.0 (ref.)	1.4 (1.0, 1.8)	1.0 (ref.)	1.7 (1.2, 2.5)	378	142	136	68	479	140
GSTM1-, *GSTT1+*	1.0 (ref.)	1.2 (0.9, 1.5)	1.0 (ref.)	1.4 (0.9, 1.9)	400	135	151	60	541	154
GSTM1+, *GSTT1-*	1.0 (ref.)	2.8 (1.6, 5.0)	1.0 (ref.)	4.8 (2.1, 11)	98	47	34	22	158	24
GSTM1-, *GSTT1-*	1.0 (ref.)	1.6 (0.9, 2.9)	1.0 (ref.)	1.4 (0.6, 3.1)	94	40	36	16	138	41
P-interaction[§]	0.03		0.09							

[*] NECC: unconditional logistic regression adjusted for age, study center, duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months); NHS: unconditional logistic regression adjusted for age in months, menopausal status at diagnosis (post, pre/dubious), DNA source, duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

[†] *P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.36

[‡] NHS analysis adjusted for age, menopausal status at diagnosis, and DNA source only, to improve stability of estimates

[§] *P*-values for interaction based on likelihood ratio test comparing unconditional logistic regression models with and without gene-talc interaction terms

Exhibit 53

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Original Contribution

Risk Factors for Epithelial Ovarian Cancer by Histologic Subtype

Margaret A. Gates*, Bernard A. Rosner, Jonathan L. Hecht, and Shelley S. Tworoger

* Correspondence to Dr. Margaret A. Gates, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115 (e-mail: nhmag@channing.harvard.edu).

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Previous epidemiologic studies suggest that the major histologic subtypes of epithelial ovarian cancer may have different risk factor profiles; however, no known prospective study has systematically examined differences in risk by subtype. The authors used Cox proportional hazards regression, stratified by histologic subtype and time period, to examine the association between ovarian cancer risk factors and incidence of serous invasive, endometrioid, and mucinous ovarian cancers in the US Nurses' Health Study (1976–2006) and Nurses' Health Study II (1989–2005). For each exposure, they calculated *P*-heterogeneity using a likelihood ratio test comparing models with separate estimates for the 3 subtypes versus a single estimate across subtypes. Analysis included 221,866 women and 721 cases with the histologies of interest (496 serous invasive, 139 endometrioid, 86 mucinous). In analyses of reproductive/hormonal exposures, the associations with age, duration of breastfeeding, age at natural menopause, and duration of estrogen use differed significantly by subtype (all *P*-heterogeneity $\leq$0.05). The associations with several nonreproductive exposures also appeared to vary by subtype, but only the association with smoking differed significantly (*P*-heterogeneity = 0.03). Results suggest that associations with several ovarian cancer risk factors vary by subtype, and these differences are consistent with known similarities between each major histologic subtype and its normal tissue counterpart.

adenocarcinoma, mucinous; carcinoma, endometrioid; cystadenocarcinoma, serous; histology; ovarian neoplasms

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; RR, incidence rate ratio.

Epithelial ovarian cancers often are analyzed as a single outcome in epidemiologic studies, despite evidence of differences in their natural history, morphology, and gene/protein expression (1–4). The most common histologic subtypes of epithelial ovarian cancer each resemble a different normal tissue in morphology and gene expression (4, 5), and previous studies suggest their etiology may differ as well. In a pooled analysis of 10 case-control studies, oral contraceptive use and parity were inversely associated with all subtypes, whereas associations with nonreproductive exposures, particularly body mass index and smoking, differed by subtype (6). Other studies have reported differences in associations with both reproductive and nonreproductive exposures for mucinous versus nonmucinous cancers (7–12).

Although these studies suggest that some associations differ by subtype, the data are inconsistent (6–10, 13, 14), and no known comprehensive, prospective analysis of differences in risk factors by histologic subtype has been published. In addition, most prior studies analyzed each subtype separately and did not report a statistical test comparing results across subtypes. We therefore used polytomous regression models to examine the association between known and suspected risk factors for ovarian cancer and incidence of the serous invasive, endometrioid, and mucinous subtypes in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII).

MATERIALS AND METHODS

Study population

The NHS was established in 1976 and the NHSII in 1989 among 121,700 US female registered nurses aged 30–55 years and 116,430 US female registered nurses aged 25–42 years, respectively. Participants completed an initial questionnaire and biennial follow-up questionnaires,

providing information on lifestyle factors and disease diagnoses. Follow-up is high in both cohorts; we obtained 95.2% of the total possible person-years through June 2006 in the NHS and 93.6% through June 2005 in the NHSII. The Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital, Boston, Massachusetts, approved both studies.

Exposure data

We obtained information on exposures of interest from the biennial questionnaires. At baseline, participants reported their birth date, age at menarche, and height. We requested information on parity, oral contraceptive use, tubal ligation, hysterectomy/oophorectomy, menopausal status, age at menopause, postmenopausal hormone use, weight, physical activity, smoking status, and family history of breast/ovarian cancer on multiple questionnaires during follow-up. In our analysis, we updated values for these covariates when new data were available and otherwise carried forward values from the previous cycle. We requested data on total duration of breastfeeding across all pregnancies in 1986 (NHS) and 1993 (NHSII) and on duration of breastfeeding for each child in 1997 (NHSII only). Information on frequency of genital talc use was collected in 1982 (NHS only).

Identification of ovarian cancer cases

We collected information on new ovarian cancer diagnoses on each questionnaire. For all reported cases, as well as deaths due to ovarian cancer identified through family members, the National Death Index (15, 16), or the US Postal Service, we obtained medical records related to the diagnosis. A gynecologic pathologist (J. H.) blinded to exposure status reviewed the medical records to confirm the diagnosis, stage, histologic type/subtype, and invasiveness (17). Our analysis included cases of epithelial ovarian cancer ($n = 885$) and primary peritoneal cancer ($n = 39$) confirmed by pathology report review and diagnosed between baseline and June 2006 (NHS) or 2005 (NHSII).

Statistical analysis

Participants accrued person-time from the return date of the baseline questionnaire until the date of ovarian cancer diagnosis, diagnosis of any other cancer (excluding nonmelanoma skin cancer), bilateral oophorectomy, pelvic irradiation, death, or the end of follow-up. At baseline, we excluded women with bilateral oophorectomy (NHS: $n = 7,669$; NHSII: $n = 2,229$), menopause due to pelvic irradiation (NHS: $n = 99$; NHSII: $n = 30$), or cancer other than nonmelanoma skin cancer (NHS: $n = 3,314$; NHSII: $n = 1,050$). In addition, we excluded women with missing data on any exposure of interest except breastfeeding duration, talc use, and family history of ovarian cancer, which were not collected at baseline, and age at natural menopause, which was missing for women with a hysterectomy before menopause. We included missing indicators for these 4 exposures in our models to avoid excluding too many women from the analysis. Participants contributed person-time only for follow-up periods for which data were complete. Furthermore, we excluded person-time ($\leq$0.3% of the total) when any continuous variable had an outlying value, using the generalized extreme studentized deviate many-outlier detection approach (18).

In analyses of reproductive/hormonal exposures, we modeled age, parity among parous women, duration of breastfeeding, duration of oral contraceptive use, age at natural menopause, and duration of postmenopausal use of unopposed estrogens as continuous variables to minimize the number of parameters in the model. We used binary variables to model menopausal status (postmenopause vs. premenopause/perimenopause), cohort (NHS vs. NHSII), and parity, tubal ligation, and hysterectomy without bilateral oophorectomy (yes/no). Because of evidence of a nonlinear association with age, we used a spline with a single knot at age 50 years to estimate linear associations with age separately for women younger than age 50 years versus 50 years of age or older.

In an alternative analysis, we modeled ovulatory years and duration of menopause instead of age, parity, duration of oral contraceptive use, and age at natural menopause. We calculated ovulatory years as current age (if premenopausal) or age at natural menopause minus age at menarche, years of oral contraceptive use, and parity (1 year per pregnancy), and we included a separate variable for total duration of breastfeeding. We calculated duration of menopause as current age minus age at natural menopause for postmenopausal women, and we coded premenopausal/perimenopausal women as 0. For women with an unknown age at natural menopause because of hysterectomy before menopause, we excluded person-time after hysterectomy.

For the nonreproductive exposures, we modeled body mass index (weight (kg)/height $(m)^2$) and physical activity (cumulative average metabolic equivalent task-hours/week) continuously, regular genital talc use ($\geq$once/week vs. $<$once/week) and family history of breast/ovarian cancer (yes/no) as binary variables, and smoking status as 2 indicator variables for past or current (vs. never) smoking. Metabolic equivalent task-hours captures both duration and intensity of activity (3 metabolic equivalent task-hours is equivalent to walking 2–2.9 mph for 1 hour (1 mile = 1.6 km)), and cumulative average levels better reflect long-term activity and minimize within-person variation. In the NHS, data on metabolic equivalent task-hours were not available until 1986; we therefore assigned all participants 0 activity from 1976 to 1986 and secondarily evaluated the association with physical activity with follow-up beginning in 1986.

We used Cox proportional hazards regression, stratified by time period, to model the incidence rate ratio and 95% confidence interval of epithelial ovarian cancer for each exposure in the NHS and NHSII combined. We then restricted the analysis to cases with serous invasive/poorly differentiated, endometrioid, or mucinous histology and used Cox proportional hazards regression, stratified by type of outcome and time period, to allow for different associations by histologic subtype (19). We used data augmentation, such that each participant had a separate observation for each subtype. We coded the event variable as 1 (failed) if

the participant was diagnosed with the histologic subtype corresponding to that data row and as 0 otherwise; cases were censored for other subtypes at the time of diagnosis.

We compared a model that assumed different associations for all exposures by histologic subtype (full model) with a model with a single estimate across subtypes for one exposure at a time (reduced model). We calculated the *P*-heterogeneity using a likelihood ratio test, with the degrees of freedom equal to the difference between the numbers of parameters in the 2 models. Using a stepwise-down approach, we set exposures with a nonsignificant *P*-heterogeneity to have a single estimate across subtypes, so that the final model estimated 3 separate associations for exposures that differed significantly by subtype and a single estimate for all other exposures. All *P* values were 2-sided and were considered statistically significant if ≤ 0.05.

We evaluated goodness of fit by calculating the area under the receiver operating characteristic curve (AUC) for all cancers and stratified by subtype. For each observation, we determined a risk score using parameter estimates from the model, and we used the risk scores to calculate the Wilcoxon rank sum test statistic W by 5-year age group t. We calculated the Mann-Whitney $U_t = W_t - \frac{m_t(m_t+1)}{2}$ and $\hat{\theta}_t = \frac{U_t}{m_t n_t}$, where $\hat{\theta}_t$ is the probability that a random case has a higher risk score than a random control within age group t. We calculated the variance of $\hat{\theta}_t$ under the alternative hypothesis (20), and we calculated the overall AUC as the weighted average of $\hat{\theta}_t$ across t with weights $= 1/\mathrm{var}(\hat{\theta}_t)$.

We did not have adequate power to examine associations with clear-cell cancers separately because of the small number of cases ($n = 48$). However, we evaluated differences between serous versus nonserous (endometrioid/mucinous/clear-cell) and mucinous versus nonmucinous (serous/endometrioid/clear-cell) cancers. In secondary analyses, we examined differences between all 4 subtypes for the reproductive exposures only.

RESULTS

Our analysis included 221,866 women with 924 incident cases of confirmed epithelial ovarian cancer (NHS: 108,870 women and 797 cases; NHSII: 112,996 women and 127 cases). Of the cases of cancer, 496 were serous invasive (54%), 139 were endometrioid (15%), and 86 were mucinous (9%). The remaining 203 cases of cancer included 48 clear cell (5% of total), 71 noninvasive serous (8%), 21 carcinosarcoma (2%), 17 mixed (2%), and 46 other/unknown (5%).

In general, baseline characteristics of cases versus noncases were similar to those expected based on previous studies of known risk factors (Table 1). NHSII participants were younger than NHS participants and were more likely to have used oral contraceptives or have had a tubal ligation, were less likely to be parous or to smoke, were more physically active, and had lower mean parity but a longer mean duration of breastfeeding among parous women.

When we compared baseline characteristics of women subsequently diagnosed with a serous invasive, endometrioid, or mucinous tumor (Table 1), we found that serous invasive cases were slightly older, had higher parity, and were more physically active than endometrioid/mucinous cases. Endometrioid cases had a longer mean duration of estrogen use (NHS only) and a higher mean body mass index (NHSII only), were less likely to be parous (NHS only) or to have smoked, and were more likely to have a family history of breast cancer. Mucinous cases had a shorter mean duration of estrogen use (NHS only) and breastfeeding and were less physically active, less likely to have had a hysterectomy, and were more likely to have regularly used talc or to currently smoke (NHS only).

The associations with age (*P*-heterogeneity <0.001), duration of breastfeeding (*P*-heterogeneity $= 0.03$), age at natural menopause (*P*-heterogeneity $= 0.05$), and duration of estrogen use (*P*-heterogeneity $= 0.009$) differed significantly by subtype, whereas other exposures (e.g., oral contraceptive use) exhibited similar associations across the 3 subtypes (Table 2). Age among women less than 50 years was more strongly associated with serous invasive (incidence rate ratio (RR) $= 1.15$ per year, 95% confidence interval (CI): 1.10, 1.19) and endometrioid (RR $= 1.12$ per year, 95% CI: 1.06, 1.17) tumors than mucinous tumors. Among women aged 50 years or older, age was associated with a modest increase in risk of serous invasive cancers, was associated with a modest decrease in risk of endometrioid tumors, and was unassociated with mucinous cancers. Duration of breastfeeding was inversely associated with all 3 subtypes, but the association was strongest for mucinous tumors (RR $= 0.43$ per year). Age at natural menopause was positively associated with the endometrioid subtype only (RR $= 1.13$ per year, 95% CI: 1.04, 1.22). Duration of estrogen use was associated with a strong increase in risk of endometrioid cancers (RR $= 1.87$ per 5-year increase, 95% CI: 1.52, 2.31) and a weaker increase in risk of the other subtypes.

Although not statistically significant, there was some evidence of heterogeneity by subtype for parity, tubal ligation, and hysterectomy; the inverse association for oral contraceptive use was similar across subtypes. A first birth was associated with a borderline significant decrease in risk of serous invasive and endometrioid cancers but was unassociated with mucinous tumors. Each additional birth significantly decreased risk of the endometrioid subtype only (RR $= 0.85$, 95% CI: 0.74, 0.99). In general, tubal ligation and hysterectomy were more strongly inversely associated with endometrioid and mucinous cancers.

In an alternative reproductive model with ovulatory years and duration of menopause, associations with number of ovulatory years (*P*-heterogeneity $= 0.04$), duration of menopause (*P*-heterogeneity <0.001), and duration of breastfeeding (*P*-heterogeneity $= 0.03$) differed significantly by subtype (Table 3). Each 1-year increase in the number of ovulatory years was associated with a significant 8% increase in risk of serous invasive and endometrioid tumors but only a 3% increase in risk of mucinous tumors.

Building on the final reproductive model, the associations with several nonreproductive exposures appeared to differ by subtype, but only smoking differed significantly (*P*-heterogeneity $= 0.03$) (Table 4). Past smoking was associated with decreased risk of endometrioid tumors (RR $=$ 0.59, 95% CI: 0.39, 0.90), whereas past/current smoking

Table 1. Baseline Characteristics of Epithelial Ovarian Cancer Cases and Noncases Among 108,870 Women in the NHS in 1976 and 112,996 Women in the NHSII in 1989

	NHS					NHSII				
	Noncases (n = 108,073)	All Epithelial (n = 797)	Serous Invasive (n = 451)	Endometrioid (n = 115)	Mucinous[a] (n = 69)	Noncases (n = 112,869)	All Epithelial (n = 127)	Serous Invasive (n = 45)	Endometrioid (n = 24)	Mucinous[a] (n = 17)
Reproductive/hormonal characteristics										
Mean										
Age, years	42	45	45	44	44	35	37	38	36	35
Duration of oral contraceptive use, months[b]	47	44	44	36	38	53	49	39	62	57
Duration of estrogen use, months[b]	34	44	43	75	20	15	0	0	0	0
Parity among parous women, no.	3.1	3.0	3.2	2.9	2.9	2.1	2.0	2.2	1.8	1.8
Duration of breastfeeding, months[c]	6	4	4	4	2	13	8	11	10	7
Ovulatory years, no.[d]	24	27	28	27	27	17	20	21	18	17
Percentage of the population										
Ever used oral contraceptives	48	38	35	38	43	83	85	87	83	82
Parous	94	90	91	82	95	70	63	67	67	53
Tubal ligation	13	8	9	7	10	16	13	18	4	6
Hysterectomy	13	14	18	10	6	4	6	7	8	0
Other characteristics										
Mean										
Body mass index, kg/m^2	24	24	24	24	23	24	26	24	29	24
Physical activity, MET-hours/week[e]	13	14	15	13	9	21	22	25	18	17
Percentage of the population										
Genital talc use >once/week[f]	28	29	29	30	40					
Past smoker	23	27	29	17	26	21	22	23	8	20
Current smoker	33	31	29	33	44	13	12	16	8	13
Family history of breast cancer	6	8	7	12	8	6	13	20	21	7
Family history of ovarian cancer[g]	3	5	6	0	19	2	1	4	0	0

Abbreviations: MET, metabolic equivalent task; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II.

[a] Includes borderline and invasive tumors.

[b] Among ever users of oral contraceptives or postmenopausal unopposed estrogens; in the NHSII, only 32 women had used unopposed estrogens at baseline.

[c] Total duration among parous women in 1986 for the NHS and 1993 for the NHSII.

[d] Current age (if premenopausal) or age at natural menopause minus (age at menarche + duration of oral contraceptive use in years + parity).

[e] Physical activity from 1986 for the NHS and 1989 for the NHSII; 3 MET-hours is equivalent to walking at an average pace of 2.0–2.9 miles/hour for 1 hour (1 mile = 1.6 km).

[f] Use among NHS participants only; collected in 1982.

[g] First collected in 1992 in the NHS and 1993 in the NHSII.

Table 2. Association Between Reproductive/Hormonal Exposures and Risk of Epithelial Ovarian Cancer, by Histologic Subtype, Among 108,870 Women in the NHS From 1976 to 2006 and 112,996 Women in the NHSII From 1989 to 2005[a]

	All Epithelial (n = 924)		Serous Invasive (n = 496)		Endometrioid (n = 139)		Mucinous (n = 86)[b]		P-Heterogeneity[c]
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	
Age among women <50 years, (per 1-year increase)[d]	1.11	1.09, 1.14	1.15	1.10, 1.19	1.12	1.06, 1.17	1.06	1.00, 1.12	<0.001
Age among women ≥50 years, (per 1-year increase)[e]	1.02	1.01, 1.04	1.04	1.02, 1.06	0.97	0.94, 1.00	1.00	0.96, 1.04	
Parous[f]	0.71	0.57, 0.89	0.73	0.53, 1.02	0.61	0.37, 1.03	1.17	0.56, 2.47	0.09
Parity among parous women[f]	0.94	0.89, 0.99	1.00	0.94, 1.06	0.85	0.74, 0.99	0.95	0.81, 1.13	
Breastfeeding (per 1-year increase)[g]	0.82	0.74, 0.91	0.84	0.73, 0.96	0.74	0.55, 1.00	0.43	0.25, 0.74	0.03
Oral contraceptive use (per 5-year increase)	0.84	0.75, 0.93	0.78	0.66, 0.91	0.77	0.58, 1.02	0.84	0.60, 1.17	0.91
Tubal ligation	0.68	0.56, 0.84	0.83	0.63, 1.09	0.59	0.34, 1.02	0.50	0.25, 1.01	0.26
Hysterectomy	0.69	0.52, 0.91	0.86	0.61, 1.20	0.68	0.39, 1.17	0.45	0.20, 0.98	0.20
Age at natural menopause (per 1-year increase)	1.03	1.00, 1.05	1.02	0.99, 1.06	1.13	1.04, 1.22	1.01	0.93, 1.10	0.05
Estrogen use (per 5-year increase)[h]	1.37	1.25, 1.50	1.28	1.14, 1.44	1.87	1.52, 2.31	1.31	0.89, 1.93	0.009

Abbreviations: CI, confidence interval; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; RR, incidence rate ratio.

[a] Estimates were adjusted for all variables in the table, plus cohort (NHS or NHSII), menopausal status (postmenopause vs. premenopause/perimenopause), missing data on breastfeeding duration (yes/no) because of noncompletion of questionnaire, and missing age at natural menopause (yes/no) because of hysterectomy prior to menopause.

[b] Includes borderline and invasive tumors.

[c] P value from likelihood ratio test comparing, for each covariate, the model with separate estimates for the serous invasive, endometrioid, and mucinous histologic subtypes with the model with a single estimate across the 3 subtypes.

[d] RR for each 1-year increase in age prior to age 50 years.

[e] RR for each 1-year increase in age at age 50 years or older.

[f] Parous: RR for 1 versus 0 children; parity among parous women: RR for each additional child after the first.

[g] Breastfeeding duration first collected in 1986 in the NHS and 1993 in the NHSII.

[h] Duration of postmenopausal use of unopposed estrogens.

was associated with a nonsignificant increased risk of mucinous cancers. Body mass index was positively associated with the endometrioid subtype (RR = 1.18 per 5 kg/m^2, 95% CI: 1.02, 1.38) but was unassociated with the other subtypes (P-heterogeneity = 0.06). There also were nonsignificant positive associations between physical activity and serous invasive cancers and between talc use and mucinous tumors. The results for physical activity were unchanged when 1986 was used as the baseline (results not shown).

For the association with all epithelial cancers, the AUC for the reproductive model (AUC = 0.624) was slightly higher than that for the ovulatory years model (AUC = 0.617), indicating that these models have similar discriminatory ability (Table 5). The goodness of fit for the reproductive model was highest for the endometrioid subtype (AUC = 0.714), intermediate for the mucinous subtype (AUC = 0.678), and lowest for the serous invasive subtype (AUC = 0.614). Adding the nonreproductive exposures improved the goodness of fit overall and for each subtype. Although the AUC for each model was based on a slightly different study population, the results were similar when we used the same population for all models (results not shown).

All results were essentially unchanged when we restricted analyses to the NHS only or excluded primary peritoneal cases (results not shown). In analyses of serous versus nonserous cancers, there were significant differences for the associations with age, parity, tubal ligation, and duration of breastfeeding but no differences for nonreproductive exposures (results not shown). When mucinous cancers were compared with nonmucinous cancers, the associations with only age, duration of breastfeeding, and number of ovulatory years differed significantly (results not shown). When we included clear-cell cancers in the reproductive model, the associations with age, parity, duration of estrogen use, and duration of breastfeeding differed significantly across the 4 subtypes (results not shown).

DISCUSSION

These results suggest that associations with several ovarian cancer risk factors differ by histologic subtype. We observed significant heterogeneity across the serous invasive, endometrioid, and mucinous subtypes for associations with both reproductive and nonreproductive exposures, including age, duration of breastfeeding, duration of estrogen use, and smoking status. There was some evidence of heterogeneity by subtype for several other exposures, including parity and

Table 3. Association Between Ovulatory Years and Other Reproductive/Hormonal Exposures and Risk of Epithelial Ovarian Cancer, by Histologic Subtype, Among 107,352 Women in the NHS From 1976 to 2006 and 112,632 Women in the NHSII From 1989 to 2005[a,b]

	All Epithelial (n = 767)		Serous Invasive (n = 397)		Endometrioid (n = 118)		Mucinous[c] (n = 80)		P-Heterogeneity[d]
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	
Ovulatory years (per 1-year increase)[e]	1.07	1.05, 1.08	1.08	1.06, 1.10	1.08	1.05, 1.11	1.03	1.00, 1.07	0.04
Duration of menopause (per 1-year increase)	1.02	1.01, 1.04	1.04	1.02, 1.06	0.96	0.93, 0.99	1.00	0.97, 1.04	<0.001
Breastfeeding (per 1-year increase)[f]	0.80	0.71, 0.89	0.85	0.73, 0.98	0.68	0.49, 0.94	0.45	0.27, 0.77	0.03
Tubal ligation	0.69	0.55, 0.85	0.86	0.65, 1.16	0.57	0.32, 1.00	0.51	0.25, 1.04	0.21
Hysterectomy	0.69	0.52, 0.92	0.77	0.53, 1.13	0.78	0.42, 1.44	0.57	0.23, 1.42	0.81
Estrogen use (per 5-year increase)[g]	1.36	1.13, 1.64	1.45	1.16, 1.81	2.33	1.53, 3.53	0.93	0.38, 2.26	0.08

Abbreviations: CI, confidence interval; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; RR, incidence rate ratio.

[a] Estimates were adjusted for all variables in the table, plus cohort (NHS or NHSII), parous (yes/no), menopausal status (postmenopause vs. premenopause/perimenopause), and missing data on breastfeeding duration (yes/no) because of noncompletion of questionnaire.

[b] Model excludes women with missing age at natural menopause because of hysterectomy prior to menopause.

[c] Includes borderline and invasive tumors.

[d] P value from likelihood ratio test comparing, for each covariate, the model with separate estimates for the serous invasive, endometrioid, and mucinous histologic subtypes with the model with a single estimate across the 3 subtypes.

[e] Current age (if premenopausal) or age at natural menopause minus (age at menarche + duration of oral contraceptive use in years + parity).

[f] Breastfeeding duration first collected in 1986 in the NHS and 1993 in the NHSII.

[g] Duration of postmenopausal use of unopposed estrogens.

body mass index, but these differences were not statistically significant.

Previous epidemiologic studies have reported differences in the risk factors for each histologic subtype of ovarian cancer, although most studies were retrospective and few reported a statistical test of differences in risk across subtypes. In a pooled analysis, parity and oral contraceptive use were inversely associated with all 4 major subtypes, although parity was most protective for endometrioid and clear-cell tumors, and breastfeeding was inversely

Table 4. Association Between Nonreproductive Exposures and Risk of Epithelial Ovarian Cancer, by Histologic Subtype, Among 108,446 Women in the NHS From 1976 to 2006 and 112,054 Women in the NHSII From 1989 to 2005[a]

	All Epithelial (n = 876)		Serous Invasive (n = 468)		Endometrioid (n = 134)		Mucinous[b] (n = 84)		P-Heterogeneity[c]
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	
Body mass index (per 5-kg/m^2 increase)	1.05	0.98, 1.12	0.97	0.88, 1.07	1.18	1.02, 1.38	0.90	0.72, 1.13	0.06
Activity (per 15-MET-hour/week increase)[d]	1.05	0.98, 1.13	1.08	0.98, 1.19	0.94	0.76, 1.16	0.82	0.61, 1.10	0.11
Talc use (≥once/week vs. <once/week)[e]	1.06	0.89, 1.28	1.06	0.84, 1.35	1.06	0.66, 1.69	1.50	0.84, 2.66	0.55
Past smoker	1.05	0.91, 1.22	1.09	0.89, 1.34	0.59	0.39, 0.90	1.54	0.94, 2.53	0.03
Current smoker	1.11	0.92, 1.35	1.14	0.88, 1.49	0.93	0.59, 1.47	1.52	0.85, 2.74	
Family history of breast cancer	1.29	1.07, 1.56	1.34	1.04, 1.73	1.94	1.24, 3.03	1.42	0.76, 2.63	0.38
Family history of ovarian cancer[f]	1.75	1.19, 2.57	1.85	1.13, 3.03	0.47	0.07, 3.39	4.50	1.76, 11.51	0.06

Abbreviations: CI, confidence interval; MET, metabolic equivalent task; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; RR, incidence rate ratio.

[a] Estimates were adjusted for all variables in the table, plus all covariates in the final reproductive model (Table 2) and variables for missing data on talc use or family history of ovarian cancer (yes/no).

[b] Includes borderline and invasive tumors.

[c] P value from likelihood ratio test comparing, for each covariate, the model with separate estimates for the serous invasive, endometrioid, and mucinous histologic subtypes with the model with a single estimate across the 3 subtypes.

[d] Cumulative average physical activity beginning in 1986 for the NHS and 1989 for the NHSII.

[e] Information on regular genital talc use available for NHS participants only; collected in 1982.

[f] Information on family history of ovarian cancer first collected in 1992 in the NHS and 1993 in the NHSII.

Table 5. AUC for Total Epithelial Ovarian Cancer and Each Histologic Subtype Among Women in the NHS From 1976 to 2006 and the NHSII From 1989 to 2005

Model	All Epithelial		Serous Invasive		Endometrioid		Mucinous[a]	
	No. of Cases	AUC	No. of Cases	AUC	No. of Cases	AUC	No. of Cases	AUC
Reproductive (Table 2)	924	0.624	496	0.614	139	0.714	86	0.678
Ovulatory years (Table 3)[b]	767	0.617	397	0.616	118	0.703	80	0.650
Reproductive + nonreproductive exposures (Table 4)	876	0.645	468	0.644	134	0.748	84	0.744
Ovulatory years + nonreproductive exposures[b,c]	731	0.643	378	0.652	114	0.746	78	0.719

Abbreviations: AUC, area under the receiver operating characteristic curve; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II.
[a] Includes borderline and invasive tumors.
[b] Excludes women with missing age at natural menopause because of hysterectomy prior to menopause.
[c] Results from this model are not shown.

associated with the serous, endometrioid, and mucinous subtypes but was most protective for mucinous cancers (6). These results, as well as the pooled associations for family history, body mass index, and smoking, were consistent with our study (6). Tubal ligation was inversely associated with serous and clear-cell cancers in the pooled analysis (6), but other studies have reported inverse associations for tubal ligation or hysterectomy and risk of endometrioid and/or mucinous tumors (8, 13, 14, 21). Age at menopause was associated with an increased risk of endometrioid tumors in a small study ($n = 41$ endometrioid cases) (22) but not in 2 other studies (7, 23), and estrogen use was more strongly positively associated with endometrioid cancers in some (24–26) but not all (13, 27) previous studies. Three studies of ovulatory years reported a positive association with nonmucinous cancers but no association with the mucinous subtype (9, 10, 14), similar to our study.

Among the nonreproductive exposures, recent physical activity was inversely associated with risk of all 4 histologic subtypes in one study, although the association was statistically significant for serous cancers only (28). Similarly, another study noted inverse associations with risk of serous, endometrioid, and mucinous tumors (29). However, prospective studies, including ours (30), generally have observed null or positive associations (31–33). Several previous studies of genital talc use, including an analysis in the NHS (34), observed a stronger positive association with serous or serous invasive cancers (35–38), although 2 studies reported no difference by subtype (39, 40) and 1 reported a positive association with mucinous tumors (38). Although our results generally are consistent with the existing literature, apparent differences, such as those for talc use, may be due to the limited number of cases of endometrioid or mucinous histology.

At one time, it was believed that the majority of epithelial ovarian cancers, regardless of histology, arose through transformation of the ovarian surface epithelium. However, growing evidence suggests a varied origin of these cancers; for example, high-grade serous carcinomas may arise in the distal fallopian tube (41–43). Morphologically, serous tumors resemble normal fallopian tube epithelium, endometrioid tumors resemble normal endometrium, and mucinous tumors resemble benign intestinal mucosa or cervical epithelium (4). In addition, there are similarities in gene expression between each subtype and its corresponding normal tissue (5).

The risk factor profiles we observed are consistent with evidence that each subtype resembles a different normal tissue. For example, parity, duration of breastfeeding, and smoking were inversely associated with risk of endometrioid tumors, whereas duration of estrogen use and body mass index were positively associated with risk. This pattern of risk factors is similar to that for endometrial cancer, which is influenced by estrogens and is positively associated with hormone-related exposures, most notably obesity and estrogen use (44). For the mucinous subtype, our results suggest that exposure to carcinogens and other chemicals (e.g., tobacco smoke or talc) may increase risk, whereas surgical procedures that decrease ovarian exposure to exogenous agents (e.g., tubal ligation or hysterectomy) may be protective. Although these results generally are not consistent with known risk factors for colon or cervical cancer (45, 46), evidence exists that smoking (47, 48) and exposure to certain chemicals (49–51) may increase risk of these cancers. The serous invasive subtype was associated with reproductive and hormonal exposures, including parity, duration of oral contraceptive use, and duration of estrogen use. Limited data are available on risk factors for fallopian tube carcinoma, although parity and tubal ligation appear to be protective (52). Information on the epidemiology of serous ovarian tumors may be informative for future research of fallopian tube primary carcinomas.

Strengths of our study include the prospective data with repeated measures for most exposures and the large combined study population. In addition, methods used in this analysis allowed for estimation of separate associations with each subtype simultaneously, as well as formal tests for differences across subtypes.

Although our analysis included a large number of epithelial cases, we had a limited number of cases with certain subtypes (e.g., clear-cell and noninvasive serous cancers). Furthermore, we classified histologic subtype based on a review of pathology reports rather than a central pathology review or immunostaining. Although this categorization likely resulted in some misclassification of histologic subtype, a validation study within the NHS found that histologic subtype based on central pathology review corresponded to

the pathology report for a high percentage of cases (17). The incomplete data for a few exposures, in particular talc use and family history of ovarian cancer, also are weaknesses because the limited data may have influenced the observed associations for these exposures. The association with talc use in our analysis differed from the association in a previous analysis of the NHS cohort (34), possibly because of a greater degree of exposure misclassification over 24 years of follow-up. However, the suggestive positive association with the mucinous subtype may reflect a longer latency period between talc exposure and development of mucinous tumors. Finally, the use of a single summary measure for certain exposures, such as physical activity, also may have limited our ability to detect an association. Additional analyses of different types/intensities of physical activity and risk of each subtype would help clarify this association.

In summary, our study provides additional evidence that associations with several ovarian cancer risk factors differ by histologic subtype and that these differences are consistent with known similarities between each subtype and a corresponding normal tissue. Differences in risk by subtype may help explain variability in the association with certain exposures across study populations, because the observed associations may differ depending on the distribution of the exposure and histologies. Future epidemiologic studies of ovarian cancer therefore should examine the histologic subtypes separately to determine whether heterogeneity in the association exists across subtypes. Analyses not taking into account differences in ovarian cancer risk by histologic subtype could be misleading.

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Author affiliations: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Margaret A. Gates, Bernard A. Rosner, Shelley S. Tworoger); Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts (Margaret A. Gates, Shelley S. Tworoger); Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts (Bernard A. Rosner); and Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts (Jonathan L. Hecht).

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Exhibit 54

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ARTICLE

Perineal Powder Use and Risk of Ovarian Cancer

Serena C. Houghton, Katherine W. Reeves, Susan E. Hankinson, Lori Crawford, Dorothy Lane, Jean Wactawski-Wende, Cynthia A. Thomson, Judith K. Ockene, Susan R. Sturgeon

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Correspondence to: Susan R. Sturgeon, DrPH, MPH, University of Massachusetts Amherst, 715 North Pleasant Street, Arnold House 407, Amherst, MA 01003 (e-mail: ssturgeon@schoolph.umass.edu).

Background Case-control studies have reported an increased risk of ovarian cancer among talc users; however, the only cohort study to date found no association except for an increase in serous invasive ovarian cancers. The purpose of this analysis was to assess perineal powder use and risk of ovarian cancer prospectively in the Women's Health Initiative Observational Study cohort.

Methods Perineal powder use was assessed at baseline by self-report regarding application to genitals, sanitary napkins, or diaphragms and duration of use. The primary outcome was self-reported ovarian cancer centrally adjudicated by physicians. Cox proportional hazard regression was used to estimate risk, adjusting for covariates, including person-time until diagnosis of ovarian cancer (n = 429), death, loss to follow-up, or September 17, 2012. All statistical tests were two-sided.

Results Among 61 576 postmenopausal women, followed for a mean of 12.4 years without a history of cancer or bilateral oophorectomy, 52.6% reported ever using perineal powder. Ever use of perineal powder (hazard ratio $[HR]_{adj}$ = 1.06, 95% confidence interval [CI] = 0.87 to 1.28) was not associated with risk of ovarian cancer compared with never use. Individually, ever use of powder on the genitals (HR_{adj} = 1.12, 95% CI = 0.92 to 1.36), sanitary napkins (HR_{adj} = 0.95, 95% CI = 0.76 to 1.20), or diaphragms (HR_{adj} = 0.92, 95% CI = 0.68 to 1.23) was not associated with risk of ovarian cancer compared with never use, nor were there associations with increasing durations of use. Estimates did not differ when stratified by age or tubal ligation status.

Conclusion Based on our results, perineal powder use does not appear to influence ovarian cancer risk.

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In 2013, it is estimated that there will be 22 240 new cases of ovarian cancer and 14 030 ovarian cancer deaths in the United States (US) alone (1). Since the 1960s, there has been speculation that the use of perineal powder is associated with ovarian cancer. In 2006, the International Agency for Research on Cancer (IARC) reviewed studies examining perineal powder use and ovarian cancer and classified talc as a possible carcinogen (2,3). The proportion of US women ever using talc powder on the perineum was estimated in 2001 to be approximately 40% (4), whereas 52% reported ever use of perineal powder in 1993–1998 within the Women's Health Initiative (WHI) (5).

The primary proposed mechanism linking perineal powder use to ovarian cancer is an inflammatory response (6). Talc particulates from perineal application have been shown to migrate to the ovaries (6), disrupting the surface ovarian epithelial tissue leading to entrapment of the talc particles within inclusion cysts (7). Furthermore, tubal ligation and/or hysterectomy, which would eliminate the pathway of talc particulates to the ovaries, are associated with reduced ovarian cancer risk (6).

A meta-analysis examining the risk of ovarian cancer among ever perineal powder users vs non-users showed odds ratios (ORs) of 1.40 (95% confidence interval [CI] = 1.29 to 1.52) for population-based case-control, 1.12 (95% CI = 0.92 to 1.36) for hospital based case-control, and 1.35 (95% CI = 1.26 to 1.46) for all case-control studies (2). More recently, a large pooled analysis found that ever use of perineal powder increased epithelial ovarian cancer risk by 24% compared with non-use (OR = 1.24, 95% CI = 1.15 to 1.33) (8). Increased risk was associated with invasive serous, endometrioid, clear cell, and borderline serous subtypes of epithelial ovarian cancer (8). However, when looking at the lifetime number of applications of perineal powder, there was no statistically significant trend for increasing applications, attributed to difficulty in recalling details of frequency and duration of perineal powder use (8).

To date there has only been one prospective study conducted examining perineal powder use and risk of ovarian cancer (9). In the Nurses' Health Study (NHS) cohort, no overall association was found between ever use of perineal powder and epithelial ovarian cancer (relative risk [RR] = 1.09, 95% CI = 0.86 to 1.37) or serous ovarian cancers (RR = 1.26, 95% CI = 0.94 to 1.69) (9). However, there was a 40% (95% CI = 1.02 to 1.91) increase in risk for serous

invasive ovarian cancer with ever perineal powder use, which comprises 86% of serous ovarian cancers in this cohort (9).

Limitations of recall bias and misclassification make it difficult to determine the true relationship between perineal powder (10), a commonly used cosmetic product, and ovarian cancer, a disease with poor survival and few known modifiable risk factors. The prior prospective cohort study, which should not be affected by recall bias, had no information on duration of use limiting interpretation. Here we expand on the available evidence by assessing perineal powder use and risk of ovarian cancer in the Women's Health Initiative Observational Study (WHI-OS). The WHI-OS is a large cohort that collected information on several application areas of perineal powder use and their respective durations of use.

Methods

Study Population

The WHI-OS enrolled 93 676 women from 40 clinical centers across the United States from 1993 to 1998 (11). Women were eligible if they were aged 50 to 79 at enrollment, postmenopausal, and planned to reside in the area for at least three years (11). Women were excluded from the WHI-OS if they were participating in another clinical trial, unlikely to survive three years due to medical conditions, or had conditions that would interfere with study participation (11). Participants completed annual mailed questionnaires to update information on risk factors and outcomes, including ovarian cancer (11). Written informed consent was obtained from participants, and all clinical centers were approved by their respective institutional review boards (11). The current analysis was approved by the University of Massachusetts, Amherst Human Subjects Review Committee.

For this analysis, participants were additionally excluded if they reported a bilateral oophorectomy or an unknown number of ovaries at baseline (n = 20 960), a history of any cancer at baseline except nonmelanoma skin cancer (n = 10 622), or were missing exposure or follow up information (n = 516). After applying the exclusion criteria, 61 576 participants with 429 adjudicated incident ovarian cancer cases remained.

Exposure Ascertainment

Perineal powder use was assessed via self-report at baseline. Participants were asked, "Have you ever used powder on your private parts (genital areas)?" Those who responded yes further indicated the duration of use with the following possible responses: less than 1 year, 1–4 years, 5–9 years, 10–19 years, or 20 or more years. For persons that reported ever use of a diaphragm, participants were asked, "Did you ever use powder on your diaphragm?" and those who responded yes further indicated duration. The third category evaluated was "Did you ever use powder on a sanitary napkin or pad?" with those responding yes also reporting duration. Each area of application variable was assessed dichotomously and the duration of use, collapsed into fewer categories because of small numbers, was assessed categorically as never, 9 years or less, or 10 or more years. A combined ever perineal powder variable and duration variable for any powder use was created; where ever use was defined as report of ever use of any of the three application categories, never was report of never use for all three categories, and duration was the maximum duration reported of any single area of application, because we could not exclude the possibility that applications were concurrent. Lastly, all possible combinations of the three application areas were assessed.

Outcome Ascertainment

Ovarian cancer cases were initially self-reported by participants in the WHI-OS on annual questionnaires. Medical records, including hospital discharge summaries and pathology reports, were requested for each self-reported case and adjudicated by a physician at the local Clinical Center and then centrally by the WHI's Clinical Coordinating Center (11).

Covariate Ascertainment

Potential covariates considered included age, race, education, alcohol servings per week, smoking status, metabolic equivalent (MET) hours per week of recreational physical activity, Body Mass Index (BMI), and self-reported family history of ovarian or breast cancer. Reproductive factors considered were age at menarche, age at menopause, age at first birth, age at last birth, parity, breastfeeding duration, history of tubal ligation, history of hysterectomy, history of irregular cycles, history of endometriosis, duration of oral contraceptive use, and duration of postmenopausal hormone use. All covariates were from baseline and were not updated.

Statistical Analysis

To estimate the association between perineal powder use and ovarian cancer, proportional hazard regression models were used. Participants contributed person-time until diagnosis of ovarian cancer, death, loss to follow-up, or September 17, 2012, whichever came first. Participants with other cancers were still considered at risk for ovarian cancer and were not censored at the time of other cancer diagnoses. Information on incident oophorectomy during follow-up was not available and thus participants were not censored in this analysis. The proportional hazards assumption was tested using weighted Schoenfeld residuals.

Covariates were included in the adjusted model according to purposeful selection, where covariates with Wald *P* values of .25 or less in age-adjusted models were entered into an initial multivariable model and then each covariate was subsequently tested individually via likelihood ratio tests in order of decreasing Wald *P* values. Variables that had *P* values of .10 or less during the backwards elimination were kept in the model until a parsimonious model was obtained. Additional variables shown in previous literature (8,9) but not statistically significant in our population were also included in the final multivariable model. Lastly, family history of breast cancer and personal history of endometriosis did not change estimates and were not included in the final multivariable model.

Models fitted included the following independent variables: 1) combined ever perineal powder use, 2) ever powder use by application area (ie, applied to genitals, applied to diaphragm, or applied to sanitary napkins), 3) duration of use by application area, and 4) application area combinations (ie, genital only, diaphragm only, sanitary napkin only, genital and sanitary napkin, genital and diaphragm, diaphragm and sanitary napkin, and all three areas of application). For duration models, test for trend was used to evaluate linear trends across duration categories by modeling the

categories as a continuous variable in the multivariable regression models.

Because powder particles may not reach the ovaries due to tubal ligation and because previous studies have shown a stronger association between powder use and ovarian cancer in women without tubal ligation (4), we separately examined women without tubal ligation. We also stratified by age at baseline, because older women may have had more potential for exposure to talc contaminated with asbestos. Additionally, associations by ovarian cancer histological subtype were evaluated. All analyses were performed using Stata v.12.1 (StataCorp, College Station, TX) and two-sided *P* values of .05 or less were considered statistically significant.

Results

The average age of the participants at baseline was 63.3 years. Participants were followed for a mean of 12.4 years; never powder users were followed for a mean of 12.2 years (range = 0.12 to 17.9 years) and ever powder users were followed for a mean of 12.6 years (range = 0.03 to 18.0). The majority of the participants were white (83.7%), had less than a college degree (56.1%), and were overweight/obese (57.2%). Approximately half (52.6%) of the population reported ever use of perineal powder. Ever powder users were heavier (27.5 kg/m^2 vs 26.5 kg/m^2, $P < .0001$) and were more likely to have used oral contraceptives (44% vs 36%, $P < .0001$) and/or diaphragms (50.8% vs 37.3 %, $P < .0001$) than never users (Table 1).

Use of powder on the genitals was associated with a 12% increase in the multivariable-adjusted hazard ratio of ovarian cancer (HR_{adj} = 1.12, 95% CI = 0.92 to 1.36), though this was not statistically significant (Table 2). Use of powder on sanitary napkins (HR_{adj} = 0.95, 95% CI = 0.76 to 1.20) or diaphragms (HR_{adj} = 0.92, 95% CI = 0.68 to 1.23) also was not associated with risk. Duration of powder use on the genitals, sanitary napkins, or on the diaphragm was not associated with ovarian cancer; P_{trend} for years of use: .67, .69, and .67 respectively. Combined ever powder use from any of the three application areas did not show an association with ovarian cancer risk (HR_{adj} = 1.06, 95% CI = 0.87 to 1.28). For combined duration of use, which was the longest duration of use among the three areas of application, there was no evidence of an association with risk of ovarian cancer [P_{trend} for years of use: .77]. Use of powder on genitals, the most common application area, for 20 or more years was not associated with increased risk of ovarian cancer compared with never users (HR_{adj} = 1.10, 95% CI = 0.82 to 1.48). In a sensitivity analysis, invasive serous ovarian cancer risk was not increased (HR_{adj} = 0.96, 95% CI = 0.65 to 1.41), even in women reporting durations of use greater than 10 years.

There was no evidence of an association between perineal powder use and ovarian cancer risk by category of application (Table 3). Combined ever powder use was not associated with individual subtypes of ovarian cancer (Table 4). The multivariable-adjusted hazard ratio for serous ovarian cancer was 1.16 (95% CI = 0.88 to 1.53). Additionally, duration of combined ever powder use was also not shown to be associated with any subtype of ovarian cancer (results not shown).

The associations of combined ever powder use and ovarian cancer did not statistically differ by tubal ligation status (results not shown). When stratified by age group at baseline, hazard estimates also did not statistically differ ($P_{interaction}$ = .37); HR_{adj} for younger than 50 to 59 years = 1.29, 95% CI = 0.91 to 1.82; HR_{adj} for those 60 to 69 years = 0.94, 95% CI = 0.70 to 1.26; and HR_{adj} for those 70 to 79 years = 1.01, 95% CI = 0.68 to 1.48. When restricted to only whites or to those who had never used oral contraceptives, results were again unchanged.

Table 1. Characteristics of postmenopausal women according to perineal powder use status (n = 61 285): Women's Health Initiative Observational Study, 1993–2012

	Never perineal powder use	Ever perineal powder use
Characteristic, n (%)	**n = 29 066**	**n = 32 219**
Race		
White	24 006 (82.6)	27 336 (84.8)
Nonwhite	4991 (17.2)	4811 (14.9)
Body mass index category, kg/m^2		
<25.0	13 056 (44.9)	12 461 (38.7)
25.0–29.9	9734 (33.5)	10 799 (33.5)
30.0 +	5935 (20.4)	8571 (26.6)
Smoking status		
Never	15 347 (52.8)	15 621 (48.5)
Past	11 481 (39.5)	14 339 (44.5)
Current	1912 (6.6)	1881 (5.8)
Duration of oral contraceptive use, y		
Never	17 877 (61.5)	17 954 (55.7)
<5	6241 (21.5)	7858 (24.4)
5 to <10	2528 (8.7)	3270 (10.2)
10 to <15	1650 (5.7)	2125 (6.6)
15+	760 (2.6)	1005 (3.1)
Diaphragm use	10 826 (37.3)	16 353 (50.8)
Tubal ligation	4929 (17.0)	5901 (18.3)
Hysterectomy	6878 (23.7)	8285 (25.7)
Family history of ovarian cancer	606 (2.1)	660 (2.1)
Parity		
0	3687 (12.7)	3769 (11.7)
1–2	9773 (33.6)	11 585 (36.0)
3–4	11 101 (38.2)	12 609 (39.1)
5+	4365 (15.0)	4098 (12.7)
Age at last birth, y		
Never had term pregnancy	6219 (21.4)	6260 (19.4)
< 20	210 (0.7)	324 (1.0)
20–29	9143 (31.5)	11480 (35.6)
30+	13 011 (44.8)	13 668 (42.4)
Duration of postmenopausal hormone use, y		
Never	13 381 (46.0)	13 880 (43.1)
<5	6498 (22.4)	7546 (23.4)
5 to <10	3783 (13.0)	4567 (14.2)
10 to <15	2688 (9.3)	3128 (9.7)
15+	2716 (9.3)	3097 (9.6)

Discussion

In this large prospective study, ever perineal powder use was not associated with ovarian cancer risk, nor was it associated with ovarian cancer when assessed by area of application, duration of use, or ovarian cancer subtype. While many case-control studies have shown an approximately 24–40% increase in risk of ovarian cancer (2,8) for powder users, we did not find evidence of this association in our large, prospective analysis.

The meta-analysis of 20 case-control studies by Langseth and colleagues found a 35% increase in the odds of epithelial ovarian

Table 2. Age and multivariable-adjusted hazard ratios of ovarian cancer by area of perineal powder application (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR (95% CI)	P_{trend} †	Multivariable HR* (95% CI)	P_{trend} †
Powder use on genitals						
Never	247	457 855	1.0 (referent)	.63	1.0 (referent)	.67
Ever‡	181	304 867	1.13 (0.93 to 1.37)		1.12 (0.92 to 1.36)	
Less than 9 years	112	173 118	1.24 (0.99 to 1.55)		1.23 (0.98 to 1.54)	
10 or more years	68	129 647	0.98 (0.75 to 1.29)		0.98 (0.75 to 1.29)	
Powder use on sanitary napkins						
Never	336	590 351	1.0 (referent)	.70	1.0 (referent)	.69
Ever‡	93	172 712	0.96 (0.76 to 1.21)		0.95 (0.76 to 1.20)	
Less than 9 years	62	114 305	0.98 (0.75 to 1.28)		0.96 (0.73 to 1.26)	
10 or more years	30	56 174	0.93 (0.64 to 1.35)		0.95 (0.65 to 1.37)	
Powder use on diaphragm						
Never	373	661 239	1.0 (referent)	.78	1.0 (referent)	.67
Ever‡	52	97 714	0.94 (0.70 to 1.25)		0.92 (0.68 to 1.23)	
Less than 9 years	35	67 468	0.93 (0.66 to 1.32)		0.91 (0.64 to 1.30)	
10 or more years	17	29 202	0.99 (0.61 to 1.60)		0.95 (0.58 to 1.56)	
Combined ever powder use§						
Never	197	361 583	1.0 (referent)	.67	1.0 (referent)	.77
Ever‡	232	404 983	1.07 (0.89 to 1.30)		1.06 (0.87 to 1.28)	
Less than 9 years	135	228 931	1.12 (0.90 to 1.39)		1.09 (0.88 to 1.36)	
10 or more years	97	173 307	1.03 (0.81 to 1.31)		1.02 (0.80 to 1.30)	

* Adjusted for: Age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m^2 (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children, missing).

† Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models; P_{trend} was estimated by modeling categories as continuous. All statistical tests were two-sided.

‡ Person-years may not add up; duration information was missing for some.

§ Combined ever powder use is the longest duration of use among the applications to genitals, sanitary napkins, and diaphragms.

Table 3. Age and multivariable-adjusted hazard ratios for ovarian cancer by combined categories of powder use (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR* (95% CI)	Multivariable HR* (95% CI)
Powder Type Used				
No powder	193	355 523	1.0 (referent)	1.0 (referent)
Only genital powder	96	158 130	1.14 (0.90 to 1.46)	1.13 (0.88 to 1.45)
Only diaphragm powder	19	42 367	0.82 (0.51 to 1.32)	0.80 (0.50 to 1.29)
Only sanitary napkin powder	28	50 051	1.04 (0.70 to 1.54)	1.01 (0.68 to 1.50)
Genital and sanitary napkin powder	55	96 173	1.09 (0.80 to 1.47)	1.08 (0.80 to 1.46)
Genital and diaphragm powder	24	29 858	1.49 (0.98 to 2.28)	1.45 (0.95 to 2.23)
Diaphragm and sanitary napkin powder	4	6858	1.06 (0.40 to 2.86)	1.02 (0.38 to 2.74)
Genital, diaphragm, and sanitary napkin powder	5	18 331	0.51 (0.21 to 1.24)	0.50 (0.21 to 1.22)

* Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models. All statistical tests were two-sided. Multivariable HR adjusted for: age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m^2 (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children missing).

cancer among ever perineal powder users compared to never-users (2), and the pooled analysis of eight case-control studies by Terry and colleagues found a 24% increase in the same group (8). Langseth and colleagues did not assess dose-response or risk among subtypes of ovarian cancer (2). Terry and colleagues assessed lifetime applications of perineal powder and found no statistically significant trend with increasing lifetime applications (8). This corroborates our results that there was no statistically significant risk with increasing duration of perineal powder use, though they were able to capture both frequency and duration (8), whereas we only had duration. Terry and colleagues found elevated risks for invasive serous, borderline serous, endometrioid, and clear cell subtypes of ovarian cancer (8), which we did not observe. One potential reason that case-control studies have found slight increases in risk is the potential for an overestimation of the true association due to recall bias, because the participants are aware of their ovarian cancer status when reporting powder

Table 4. Age and multivariable-adjusted hazard ratios for combined ever powder use by subtype of ovarian cancer (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR* (95% CI)	Multivariable HR* (95% CI)
Serous†				
Never	87	355 523	1.0 (referent)	1.0 (referent)
Ever	117	404 983	1.18 (0.89 to 1.56)	1.16 (0.88 to 1.53)
Serous Invasive				
Never	80	355 523	1.0 (referent)	1.0 (referent)
Ever	105	404 983	1.16 (0.87 to 1.55)	1.13 (0.84 to 1.51)
Mucinous				
Never	12	355 523	1.0 (referent)	1.0 (referent)
Ever	13	404 983	0.98 (0.44 to 2.14)	1.03 (0.47 to 2.27)
Endometrioid				
Never	13	355 523	1.0 (referent)	1.0 (referent)
Ever	20	404 983	1.39 (0.69 to 2.79)	1.29 (0.64 to 2.61)
Other				
Never	47	355 523	1.0 (referent)	1.0 (referent)
Ever	54	404 983	1.04 (0.71 to 1.54)	1.04 (0.70 to 1.54)

* Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models. All statistical tests were two-sided. Multivariable HR adjusted for: age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m^2 (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children missing).

† Includes borderline cancers.

exposure. The prospective nature of our study would eliminate the potential for recall bias. Additionally, the case-control studies tended to have a younger population than our study, which included both premenopausal and postmenopausal ovarian cancers (2,8), whereas the WHI cohort consisted only of postmenopausal ovarian cancers. Ovarian cancer that occurs prior to menopause may have a different etiology than ovarian cancer occurring afterwards.

We found similar results to that of the NHS, the only other prospective cohort, which had a similar sample size and number of ovarian cancer cases to our study. Ever use of perineal powder did not appear to be associated with ovarian cancer in the NHS (9), similar to our findings. The results of Gertig and colleagues were also null for use on the genitals and for use on sanitary napkins (9). Additionally, neither our study nor the NHS found associations with serous ovarian cancer, endometrioid, or mucinous ovarian cancers, although subgroup sample size may have reduced statistical power to test these associations. In contrast to our results, the study by Gertig and colleagues found a 40% increase in invasive serous ovarian cancer among ever powder users compared with never powder users (9).

Strengths of our study included large sample size with a substantial number of ovarian cancer cases, a prospective cohort design, good case ascertainment, and detailed information on most ovarian cancer risk factors. We also had information on duration of powder use, qualifiers not available in several earlier studies, including the previous cohort study (2,8,9).

One potential limitation of our analyses includes a lack of information regarding oophorectomy after baseline, which would result in the inclusion of some women not at risk for ovarian cancer in the analytical cohort. However, the impact was likely to be minor, as a previous study in the WHI-OS had reported the number of persons with incident bilateral oophorectomies to be less than 250 (out of more than 90 000 participants) during nearly eight years of follow-up (12). While the prospective nature of the study design eliminates recall bias, it does not eliminate potential for nondifferential misclassification of the exposure. Women still needed to recall past perineal powder use and duration and thus may have trouble recollecting specifics regarding the use of perineal powder, leading to a bias toward the null. Information regarding powder use was not collected after baseline, and there is potential for never users to begin using powder; however, this is unlikely because the women are postmenopausal, reducing need to use perineal powder on diaphragms or sanitary napkins. We also had no specific data regarding the frequency of powder use in our sample. Frequency of use, as well as duration may influence ovarian cancer risk. We may have been comparing long-term infrequent users with short-term frequent users. If we had frequency of use in addition to the duration, we could have looked at intensity of use, which may be more accurate, and shown a dose response relationship. However, Terry and colleagues did not find a dose response relationship either when taking into account frequency and duration (8).

When restricted to women without tubal ligation status, the estimates for the association between combined ever perineal powder use and ovarian cancer were not increased. While some studies have found stronger associations between powder use and ovarian cancer in women that have not undergone a tubal ligation (4), the results from our study did not support this previous finding. The pooled analysis (8) and the NHS cohort (9) also did not find evidence of stronger associations in women without tubal ligations.

While we had information on duration of use, it is unknown during which years the perineal powder was used. Talc powder had potential for asbestos contamination (13) until 1976, when the Cosmetic, Toiletry, and Fragrance Association required all cosmetic talc products to be free of asbestos (14). Therefore, those using powder prior to 1976 may have been potentially exposed to asbestos, a known carcinogen. The pooled analysis and meta-analysis also included case-control studies not within the United States

(2,8), which potentially have different regulations regarding perineal powder and earlier studies that may have been more likely to include exposure to contaminated perineal powder (2). However, risk estimates in more recent studies are similar to earlier studies (2), reducing the likelihood that confounding by asbestos is driving the findings. Additionally, assuming older women in the cohort could have been exposed longer to perineal powder with potential contamination compared with younger women, we did not see statistically significant differences in risk when stratified by age group, further suggesting asbestos contamination is not a likely explanation.

The WHI-OS queried general perineal powder use rather than talc powder use, and we had no specific information regarding the content of talc in products used, which the previous literature reviewed by IARC suggested to be the possible carcinogen of concern (2). However, the NHS cohort and most studies included within the pooled analyses asked about general perineal powder use as well (2,8,9). In summary, perineal powder use did not appear to be associated with ovarian cancer risk in this large sample of postmenopausal women, even with use for long durations.

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Notes

WHI Investigators:

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, MD) Jacques Rossouw, Shari Ludlam, Dale Burwen, Joan McGowan, Leslie Ford, and Nancy Geller.

Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, WA) Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg.

Investigators and Academic Centers: (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) JoAnn E. Manson; (MedStar Health Research Institute/Howard University, Washington, DC) Barbara V. Howard; (Stanford Prevention Research Center, Stanford, CA) Marcia L. Stefanick; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Arizona, Tucson/Phoenix, AZ) Cynthia A. Thomson; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker.

Women's Health Initiative Memory Study: (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker.

Affiliations of authors: Division of Biostatistics and Epidemiology, University of Massachusetts Amherst, Amherst, MA (SCH, KWR, SEH, LC, SRS); Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (SEH); Department of Preventive Medicine, Stony Brook University School of Medicine, New York, NY (DL); Department of Social and Preventive Medicine, University at Buffalo, SUNY, Buffalo, NY (JWW); Health Promotion Sciences Division, College of Public Health and University of Arizona Cancer Center, Tucson, AZ (CAT); Division of Preventive and Behavioral Medicine, University of Massachusetts Medical School, Worcester, MA (JKO).

Exhibit 55

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Douching, Talc Use, and Risk of Ovarian Cancer

NL Gonzalez[1], **KM O'Brien**, **AA D'Aloisio**, **DP Sandler**, and **CR Weinberg**

[1](a)Biostatistics and Computational Biology Branch, National Institute of Environmental Health Sciences, Research triangle Park, NC; (b)Social & Scientific Systems, Inc., Durham, NC; and (c)Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Abstract

Background—Douching was recently reported to be associated with elevated levels of urinary metabolites of endocrine disrupting phthalates, but there is no literature on douching in relation to ovarian cancer. Numerous case-control studies of genital talc use have reported an increased risk of ovarian cancer, but prospective cohort studies have not uniformly confirmed this association. Behavioral correlation between talc use and douching could produce confounding.

Methods—The Sister Study (2003–2009) enrolled and followed 50,884 women in the US and Puerto Rico who had a sister diagnosed with breast cancer. At baseline participants were asked about douching and talc use during the previous 12 months. During follow-up (median of 6.6 years) 154 participants reported a diagnosis of ovarian cancer. We computed adjusted hazard ratios (HR) and 95% confidence intervals (CI) for ovarian cancer risk using the Cox proportional hazards model.

Results—There was little association between baseline perineal talc use and subsequent ovarian cancer (HR: 0.73 CI: 0.44, 1.2). Douching was more common among talc users (OR: 2.1 CI: 2.0, 2.3), and douching at baseline was associated with increased subsequent risk of ovarian cancer (HR: 1.9 CI: 1.2, 2.8).

Conclusions—Douching but not talc use was associated with increased risk of ovarian cancer in the Sister Study.

Keywords

ovarian cancer; talc; douching; phthalates

Introduction

Cancer of the ovary is the most lethal gynecological cancer in women, and its etiologies remain poorly understood. In 2015, there were an estimated 21,290 new cases and 14,180 ovarian cancer deaths among women in the United States (1). Family history of ovarian or breast cancer is a major risk factor. Nulliparity is also associated with increased risk of

Corresponding Author Contact Information: Dr. Clarice Weinberg, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, P.O. Box 12233, Research Triangle Park, NC 27709, weinberg@niehs.nih.gov.

ovarian cancer, whereas tubal ligation and oral contraceptive use are reportedly associated with reduced risk (2).

Genital talc use and douching could plausibly introduce particles and toxicants into the upper reproductive tract and increase the risk of cancers and infections. Talc particles have been found embedded in cervical and ovarian tumors (3). Some douching products are known to contain phthalates, which disrupt endocrine pathways and could influence ovarian cancer risk through hormone disruption (4). A recent analysis of data from the National Health and Nutrition Examination Survey found an association between douching and urinary concentrations of phthalates (5). Douching has also been associated with adverse health effects and reproductive problems such as pelvic inflammatory disease and ectopic pregnancy (6), as well as decreased fertility (7).

To the best of our knowledge, no existing studies have investigated the association between douching and ovarian cancer, but talc use was associated with ovarian cancer in many case-control studies (8–13). A meta-analysis of 14 population-based, case-control studies (14) and a large, pooled case-control analysis (15) both reported positive associations between genital talc use (ever vs. never) and ovarian cancer. The only prospective studies to examine talc and ovarian cancer (16, 17) found no strong associations overall, but one observed increased risk for invasive serous ovarian cancer, specifically (17). In this study we investigate the association between ovarian cancer and both douching and talc use, using prospective data from the Sister Study cohort.

Methods

The Sister Study, launched in 2003, enrolled 50,884 women across the United States and Puerto Rico. Enrollees were aged 35 to 74 years and had never had breast cancer but each had a full or half-sister who had been diagnosed with breast cancer. More than one sister per family could participate.

After excluding participants who had bilateral oophorectomies (N=9,023) or ovarian cancer (N=167) prior to enrollment or who had no follow-up information (N=40), we included 41,654 participants in this analysis. As of July 2014 (median follow-up 6.5 years), 154 incident ovarian cancer cases had occurred. We included tumors of the ovary (N=135), fallopian tubes (N=7), peritoneum (N=4), or of uncertain origin but likely from one of the three aforementioned primary sites (N=8). The Institutional Review Boards of the National Institute of Environmental Health Sciences and the Copernicus Group approved this study and all participants provided written consent.

Participants completed computer-assisted telephone interviews, which included questions about reproductive history (including any oophorectomies), health conditions, and lifestyle factors. Participants also completed a self-administered questionnaire about personal care products used in the 12 months prior to enrollment, which included questions about frequency of douching and about genital talc use in the form of powder or spray applied to a sanitary napkin, underwear, diaphragm, cervical cap, or vaginal area. Response categories were: did not use, used less than once a month, used 1–3 times per month, 1–5 times per

week, or more than 5 times per week. Because most members of the cohort reported not douching and not using talc, we used dichotomous use/nonuse variables for analysis.

Updated information on oophorectomies was collected in follow-up questionnaires administered every 2–3 years. We ascertained information on any new cancers via an annual health update and the follow-up questionnaires and were able to confirm 96 of the ovarian cancer cases using medical records (N=87) or death certificate/National Death Index data (N=9). For the remaining 58 cases, we relied on information provided by the participant herself (N=52) or her next of kin (N=6). Among women with available medical records who self-reported ovarian cancer, 90% were confirmed.

There were five eligible cases with an unknown exact age at diagnosis. For them, we imputed an age midway between their last ovarian cancer-free follow-up interview and their age at the time we were notified of the diagnosis (or death). Although we did not genotype women directly for *BRCA1* or *BRCA2* mutations, we asked each woman in her baseline interview whether she had ever been tested and, if so, what the result of those tests were. For the purposes of this analysis, a woman was treated as *BRCA1/2* mutation positive if 1) she had a positive test or 2) she had a sister with a known positive test and she had no known negative test.

Statistical Analyses

We computed adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the association of talc use and douching with ovarian cancer risk using Cox proportional hazards models, with age as the primary time scale. Follow-up lasted from age at baseline until age at diagnosis of ovarian cancer. Follow-up time was censored at their age of bilateral oophorectomy after baseline, death, or last contact. Because some participants had sisters who also enrolled in the cohort, we used generalized estimating equation methods to calculate robust variances to account for family clustering. We evaluated proportionality assumptions of the Cox model by assessing the improvement in goodness-of-fit provided by including an age-by-factor interaction term.

In addition to the main effect, we evaluated the joint effect of both douching and using talc. We classified participants into four categories: neither exposure, talc use exclusively, douching exclusively, or both exposures. We also carried out a number of stratified analyses. We stratified by reproductive factors such as menopausal status, parity, hysterectomy, and tubal ligation to explore possible effect modification (10, 13). We tested for differences across strata using the p-value for an exposure-by-modifier interaction term.

We selected potential confounders or effect modifiers of the association between ovarian cancer and the exposures of interest in this analysis *a priori* based on assumed causal relationships among the covariates (18), and included: patency (yes/no blockage of reproductive tract by tubal ligation or hysterectomy), menopausal status (pre- or postmenopausal), duration of oral contraceptive use (none, <2 years, 2–<10 years, 10 or more years), parity (yes/no), race (non-Hispanic white, non-Hispanic black, Hispanic or other), and body mass index (BMI; <25, 25–29.9, or >30 kg/m^2), all of which were fixed at baseline levels.

We conducted six sensitivity analyses. In the first, we restricted to the 96 cases confirmed by medical record or death certificate/National Death Index data. For our second sensitivity analysis we looked for evidence of etiologic heterogeneity by further restricting this pool to medically confirmed cases with serous ovarian cancer (N=49). For our third sensitivity analysis, we included all 154 eligible ovarian cancer cases as well as 5 additional cases that had unknown ages at diagnosis and pre-baseline oophorectomies (N=159 cases total). We did this to examine the influence of our assumptions about the relative timing of their oophorectomies versus their ovarian cancer diagnoses. We further examined the influence of imputing age at diagnosis in our fourth sensitivity analysis by excluding the 5 cases with imputed diagnosis ages but intact ovaries (N=149 cases total). For our fifth sensitivity analysis, we excluded participants from families known to carry *BRCA* mutations (N=347 exclusions, including 10 cases) since the lifetime risk of ovarian cancer for individuals with a BRCA1/2 mutation is substantially higher (19) and the etiology may be different. Lastly, we conducted analyses excluding the first year of follow-up, to minimize the possibility that symptoms of undiagnosed ovarian cancer were leading participants to use douche or talc. All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC) and using the Sister Study data release version 4.1.

Results

Table 1 summarizes characteristics of cases and non-cases at baseline. Most participants were non-Hispanic white (84%), and most were postmenopausal (56%). Women who later became cases were somewhat older (mean 57.8 versus 54.8), more often white, and more often nulliparous. Cases were also more likely to have a first-degree family history of ovarian cancer and more than one first-degree relative with breast cancer. They were also more likely to carry a deleterious mutation in *BRCA1* or *BRCA2*. While ever/never use of oral contraceptive was similar across cases and non-cases, the distribution of duration of use differed. More non-cases (26%) than cases (16%) had used oral contraceptive for more than 10 years. Compared to women who neither douched nor used talc, women who douched were more likely to be non-Hispanic black (23% vs. 6%) and to have less than a college degree (62% vs. 44%) and women who used talc were more likely to have a BMI over 30 kg/m^2 (41% vs. 25%; eTable).

Douching in the 12 months prior to study enrollment was reported by 13% of non-cases and 20% of cases (Table 2). Talc use in the 12 months prior to study enrollment was reported by 14% of non-cases and 12% of cases. Only 7 cases (5%) reported both douching and talc use.

Ever douching during the 12 months prior to study entry was associated with increased ovarian cancer risk (adjusted HR: 1.8, 95% CI: 1.2, 2.8; Table 2). By contrast, talc use during the 12 months prior to study entry was associated with reduced risk after the same confounder adjustments (HR: 0.73 CI: 0.44, 1.2) and there was a negligible change in the estimated effect with additional adjustment for douching (HR: 0.70 CI: 0.42, 1.1). We observed no proportional hazards assumption violations for any of the examined models.

Douching with no talc use was also associated with increased risk of ovarian cancer compared with use of neither talc nor douching (adjusted HR: 1.9 CI: 1.2, 2.9), which is

similar to the overall effect estimate of douching. There was an inverse association between exclusive talc use and ovarian cancer, and a positive association for douching and talc use combined (HR: 1.8, CI: 0.81, 3.9). There was no evidence for interaction on a multiplicative (p=0.39) or additive (p=0.72) scale.

To explore effect modification, we performed analyses stratified by a number of reproductive factors including tubal ligation status, hysterectomy status, menopause status, and parity (Figure). We also stratified by patency to see if blockage of access to the ovaries by either tubal ligation or hysterectomy might modify the association between ovarian cancer and douching or talc use. For all stratifications, there were no modifications of effect estimates for either douching or talc use (all heterogeneity p-values >0.05).

HRs for talc use differed little in the first five sensitivity analyses, showing a HR change no greater than 0.04. By contrast, exclusion of ovarian cancers without medical record or death certificate confirmation (by censoring their follow-up at the reported diagnosis age) attenuated the association between douching and ovarian cancer (HR: 1.1, CI: 0.62, 2.1). Likewise, restriction to medically confirmed serous ovarian cancer also attenuated effect estimates (HR: 1.4 CI: 0.64, 3.2). However, ovarian cancer cases who had reported that they douched were substantially less likely to have a medical record available (40%) than ovarian cases who did not douche (69%), suggesting that medical records were informatively missing, biasing results based on the restricted analysis. There was very little change in douching effect estimates when excluding the five cases with uncertain diagnosis dates or including the five women reporting oophorectomies before the diagnosis of ovarian cancer. Exclusion of known positive *BRCA1/2* families slightly strengthened the association between douching and ovarian cancer (HR: 1.9, CI: 1.3, 2.9). In our sixth sensitivity analysis, exclusion of the first year of follow-up time resulted in negligible changes in the HRs for douching and talc use (HR: 1.8, CI: 1.2, 2.8 and HR: 0.86, CI: 0.52, 1.4 respectively).

Discussion

In this large prospective cohort, which gave rise to 154 incident cases of ovarian cancer, there was a positive association between douching and incident ovarian cancer. Talc use was associated with a slight reduction of ovarian cancer risk. Our study of ovarian cancer grouped together all cancers designated as ovarian (88%), fallopian (5%), peritoneal (3%), or those designated as uncertain but either ovarian, fallopian, or peritoneal (5%). With recent literature suggesting that most cancers classified as ovarian likely originated in the fallopian tubes (20), we felt that this grouping was appropriate.

Interest in talc as a carcinogen arose because of its chemical similarity to asbestos, which has been previously linked to ovarian cancer (21). One challenge with studying talc is that the chemical formulation of talc has changed over time (9), and not all powders contain the mineral talc (e.g. cornstarch-based products). Previous case-control studies have noted evidence for a positive association (8–13), with some evidence that the effect is strongest in premenopausal women (13). Given these results, the biological plausibility, the rarity of the exposure, and imprecision of estimates, we cannot exclude a small increase in risk

associated with talc use, despite our inverse findings. Then again, with the exception of the finding that talc use was positively associated with serous ovarian cancer in the Nurses' Health Study (17), the prospective studies have not provided evidence supporting an association between talc use and ovarian cancer overall (17) or between talc use and ovarian cancer overall among post-menopausal women (16).

The numbers for the Sister Study as a whole given in Table 2 reveal an odds ratio of 2.1 (CI: 2.0, 2.3) for douching in relation to talc use. Thus, the two practices are correlated. If douching is a risk factor for ovarian cancer, some of the earlier reports on talc could have been subject to confounding bias. However, the one case-control study that did include douching as a covariate still observed a positive association between talc use and ovarian cancer risk (8). Another factor that may contribute to our null findings is that we categorized the exposure based on the 12 months prior to enrollment as a dichotomous ever/never factor rather than a quantitative measure of total applications, as has been done in previous studies.

Because Sister Study participants all have a first-degree family history of breast cancer, they are more likely than the general population to develop ovarian cancer (estimated observed/expected number of cases = 1.6 based on SEER rates). We also note that, by design, we excluded women with a previous history of breast cancer, thereby discounting some individuals who were at increased risk for ovarian cancer. While these selective factors may limit generalizability, there is no clear mechanism by which they would bias the estimated effect of talc use or douching on ovarian cancer.

Our review of the literature suggests that our study is the first to examine the association between douching and ovarian cancer. This association could reflect uncontrolled confounding by behavioral factors we have not captured well. For example, women may be more likely to douche if they are prone to infections or other reproductive health problems that could themselves be related to ovarian cancer.

If the association is causal, it could be related to the recently reported positive association between douching and higher urinary levels of phthalate metabolites observed in National Health and Nutrition Examination Survey participants (5). Phthalates are endocrine-disrupting chemicals and may be harmful to the fallopian tubes or the ovaries (22). In an animal study, exposure to di-(2-ethylhexyl) phthalate at 500 and 2,000 mg/kg demonstrated ovarian toxicity through decreased progesterone and increased apoptosis in granulosa cells (23). Further, ovarian cancer cell lines have been found to increase cell proliferation and to up-regulate cell-cycle regulatory genes following treatment with di-n-butyl phthalate (24). We did not collect detailed information about specific products used in douching, so we are unable to estimate exposure to individual phthalates.

Douching could also force tissue, menstrual fluids, or foreign materials up the reproductive tract, resulting in inflammation (e.g. pelvic inflammatory disease (6)) or infection of the fallopian tubes or ovaries themselves. This inflammation and infection could also contribute to ovarian cancer risk, as supported by the observed positive association between pelvic inflammatory disease and ovarian cancer (25).

If the association is causal and related to the transfer of xenobiotics into the upper reproductive tract, we would expect to see a stronger association in women with both a uterus and patent fallopian tubes. However, the evidence in our data appeared to be driven by the subcohort of women with hysterectomy and/or tubal ligation (Figure).

Since our study was prospective in nature, it is robust to potential differential reporting bias as exposures are recorded prior to development of cancer. Another important strength of the study was that we controlled for many potentially confounding factors.

An important limitation of our study is that we collected douching and talc information on individuals for the year prior to study entry and have not accounted for the latency of ovarian cancer, which is likely to be long (26). If latency is 15 to 20 years, douching habits at baseline do not accurately reflect the period of risk, although women who douched at baseline are likely to have been douching for a substantial amount of time before that as well. Also, given that there have been health advisories against douching because of its other potential risks, participants who douched in the past may have stopped douching and would be misclassified. Thus, the relative risk for douching in relation to ovarian cancer could be underestimated. Future studies that ascertain a complete history of douching are warranted.

Although the baseline questionnaire did ask women about their use of douche and talc between the ages 10 and 13, very few women responded yes to douching (2%), and we were unable to make use of those data. By contrast, talc use during ages 10–13 had a prevalence of 18% in the cohort, but there was no detectable effect of pre-pubertal talc use on risk (HR: 1.1 CI: 0.74, 1.7).

Exposure information was very complete, with only 832 participants (2%) missing the personal care products questionnaire entirely, and an additional 655 and 1,188 missing data for the questions about douching or talc use, respectively. However, for approximately 37% of cases we have not yet received medical records to confirm the diagnosis. We found that medical record retrieval was differential by exposure, with a lower proportion with medical records among women who douched than among women who did not. This informative missingness mathematically contributed to the substantial attenuation in the HR estimate for the association between vaginal douching and ovarian cancer when we restricted to cases with medical record confirmation. Medical record retrieval for ovarian cancer began only recently, and women with cancers diagnosed early in follow-up are more likely to be missing medical record information. Some of the unconfirmed diagnoses may be confirmed later via medical records or the National Death Index.

In this large, prospective study, we did not observe an association between recent talc use and ovarian cancer risk, but did find a strong positive association between douching and ovarian cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

BMI	body mass index
CI	confidence interval
HR	hazard ratio

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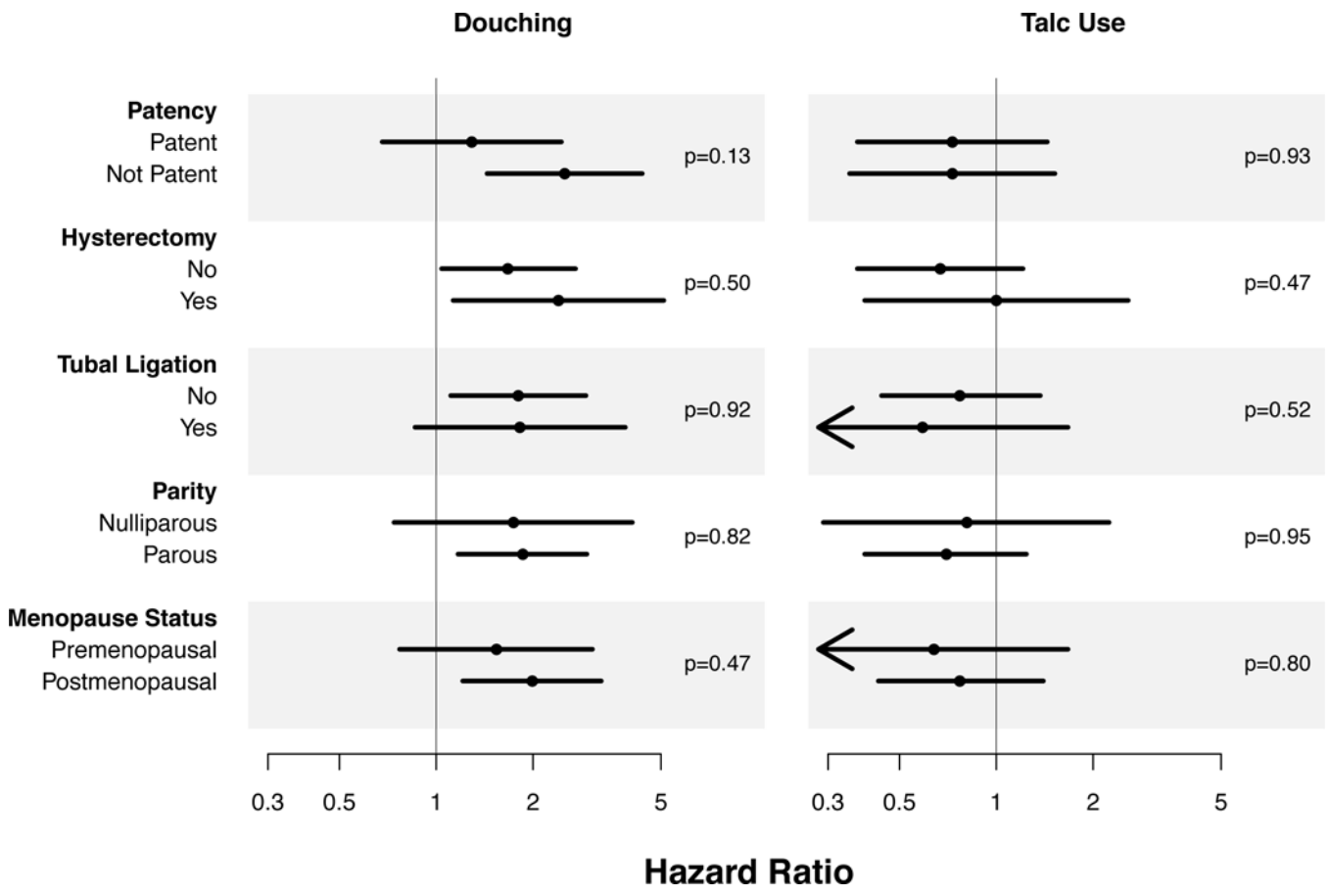

Figure.

Effect estimates of douching and talc use in the Sister Study when stratified by multiple reproductive factor, adjusted for race, body mass index, parity, duration of oral contraceptive use, baseline menopause status, and patency. The reported heterogeneity p-values are for tests of an exposure-by-modifier interaction term.

TABLE 1

Baseline characteristics of the Sister Study cohort (2003–2009)[a]

	Non-Cases (N=41,500)	Ovarian Cancer Cases (N=154)
Race; N (%)		
Non-Hispanic White	34,745 (84)	138 (90)
Non-Hispanic Black	3,598 (9)	9 (6)
Hispanic	2,076 (5)	5 (3)
Other	1,068 (2)	2 (1)
Education; N (%)		
High school or less	6,001 (14)	24 (15)
Some college	13,556 (33)	49 (32)
Bachelor's degree	11,579 (28)	46 (30)
Graduate degree	10,354 (25)	35 (23)
BMI; N (%)		
<25.0 kg/m^2	16,610 (40)	51 (33)
25–29.9 kg/m^2	13,012 (31)	51 (33)
≥ 30 kg/m^2	11,866 (29)	52 (34)
Menopausal Status; N (%)		
Premenopausal	15,238 (37)	40 (26)
Hysterectomy with ovaries retained	2,996 (7)	8 (5)
Postmenopausal	23,239 (56)	106 (69)
Hysterectomy; N (%)		
No	34,481 (83)	120 (78)
Yes	6,995 (17)	34 (22)
Tubal Ligation; N (%)		
No	29,511 (71)	115 (75)
Yes	11,973 (29)	39 (25)
Oral Contraception		
Duration of Use; N (%)		
None	6,452 (16)	25 (16)
<2 years	6,382 (15)	37 (24)
2–10 years	17,769 (43)	67 (44)
10 years or more	10,865 (26)	25 (16)
Parity; N (%)		
No live births	7,657 (18)	37 (24)
1 or more live births	33,816 (82)	116 (76)
First Degree Family History of		
Ovarian Cancer; N (%)		
No	40,149 (97)	138 (90)
>1 first-degree relative	1,322 (3)	16 (10)
Breast Cancer; N (%)		

	Non-Cases (N=41,500)	Ovarian Cancer Cases (N=154)
1 affected sister	31,291 (75)	109 (71)
>1 sister or sister+mom	10,207 (25)	45 (29)
BRCA1/2 mutation status; N (%)		
No known mutation	41,163 (99)	144 (94)
Known mutation	337 (1)	10 (6)

Missing values: Race (13 non-cases), education (10 non-cases), BMI (12 non-cases), menopausal status (27 non-cases), tubal ligation (16 non-cases), hysterectomy (24 non-cases), oral contraception use (32 non-cases), parity (1 case, 27 non-cases), ovarian cancer family history (29 non-cases), breast cancer family history (2 non-cases).

[a]Excludes women who were diagnosed with ovarian cancer before completion of the baseline interview (N=167), women who had a bilateral oophorectomy before the baseline interview (N=9,005), and women lost to follow-up (N=40).

TABLE 2

Exposure prevalence and hazard ratios for their associations with ovarian cancer in the Sister Study

	Non-cases N=41,500	Ovarian cases N=154	Fully Adjusted Hazard Ratio[a]
Douching past 12 months			
No	34,653 (87)	121 (80)	1.00
Yes	5,364 (13)	30 (20)	1.84 (1.2, 2.8)
Talc use past 12 months			
No	33,770 (86)	130 (88)	1.00
Yes	5,718 (14)	17 (12)	0.73 (0.44, 1.2)
Douched and used talcum powder past 12 months			
Neither	29,596 (76)	106 (72)	1.00
Talc use/no douching	4,399 (11)	10 (7)	0.60 (0.31, 1.1)
Douching/no talc use	3,936 (10)	23 (16)	1.9 (1.2, 2.9)
Both	1,237 (3)	7 (5)	1.8 (0.81, 3.9)

Missing values: Douching (3 cases, 1,483 non-cases), talc use (7 cases, 2,012 non-cases)

Adjusted for race, body mass index, parity, duration of oral contraceptive use, baseline menopause status, and patency.

[a]Adjusted for race, body mass index, parity, duration of oral contraceptive use, baseline menopause status, and patency. The reported heterogeneity p-values are for tests of an exposure by modifier interaction term.

Exhibit 56

Draft Screening Assessment

Talc
($Mg_3H_2(SiO_3)_4$)

Chemical Abstracts Service Registry Number
14807-96-6

Environment and Climate Change Canada
Health Canada

December 2018

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc. The Chemical Abstracts Service Registry Number (CAS RN[1]) for talc is 14807-96-6. This substance is among those substances identified as priorities for assessment as it met categorization criteria under subsection 73(1) of CEPA.

Talc is a naturally occurring mineral. According to information reported under section 71 of CEPA and publically available information, in 2011 talc was manufactured in Canada in quantities ranging between 50 to 75 million kg, and in 2016, approximately 100 million kg of talc was imported. In Canada talc is used in adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials; ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products, mixtures, and manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment. The major uses in Canada align with major global uses of talc. Talc is an ingredient in self-care products and is a permitted food additive. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics. High-purity talc is used in cosmetics, while lower-grade talc is used in commercial applications.

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach. The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of new PNEC values when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environment concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment. The ERC-I identified talc as having a low potential to cause ecological harm.

Considering all available lines of evidence presented in this draft screening assessment, there is a low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(*a*) or (*b*) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or

[1] The Chemical Abstracts Service Registry Number (CAS RN) is the property of the American Chemical Society, and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior written permission of the American Chemical Society.

may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Talc has been reviewed internationally by other organizations, including the International Agency for Research on Cancer (IARC) and the Danish Environmental Protection Agency. These assessments informed the human health risk assessment.

No critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and self-care products is not of concern. Inhalation exposure from industrial and commercial uses of talc was not identified to be of concern for human health given the limited number of sites producing and processing talc in Canada. Rather, the focus of the assessment is on inhalation and perineal exposure to certain self-care products containing cosmetic- or pharmaceutical-grade talc.

With respect to inhalation exposure, non-cancer lung effects were identified as a critical health effect for risk characterization on the basis of United States National Toxicology Program studies conducted with rats and mice exposed to cosmetic-grade talc. There is potential for inhalation exposure to talc powder during the use of certain self-care products (e.g., cosmetics, natural health products, non-prescription drugs formulated as loose powders). Self-care products formulated as pressed powders (e.g., face makeup) are not of concern. Margins of exposure between air concentrations following the use of dry hair shampoo and critical lung effects observed in animal studies are considered adequate to address uncertainties in the health effects and exposure databases. Margins of exposure between air concentrations following the use of loose powders (e.g., body powder, baby powder, face powder, foot powder) and critical lung effect levels observed in animal studies are considered potentially inadequate to address uncertainties in the health effects and exposure databases.

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs), a potential concern for human health has been identified.

Based on the available information, it is proposed that there is potential for harm to human health in Canada at current levels of exposure. Therefore, on the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(*c*) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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1. Introduction

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc to determine whether this substance presents or may present a risk to the environment or to human health. This substance was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA (ECCC, HC [modified 2017]).

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach (ECCC 2018). The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of a new PNEC value when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environmental concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment.

With respect to human health, this draft screening assessment includes the consideration of information on chemical properties, environmental fate, hazards, uses, and exposures, including additional information submitted by stakeholders. Relevant data were identified up to August 2018. Empirical data from key studies, as well as results from models, were used to reach proposed conclusions. Talc has been reviewed internationally through the International Agency for Research on Cancer (IARC) Monographs Programme, United States Environmental Protection Agency (U.S. EPA), the Joint Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Danish Environmental Protection Agency (Danish EPA). Talc was also assessed by the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany and the Cosmetic Ingredient Review (CIR) Expert Panel. These evaluations and reviews were used to inform the health effects characterization in this screening assessment. This assessment focuses on health effects associated with cosmetic-grade talc and not on potential impurities, such as asbestos. Engineered nanomaterials composed of or containing talc are not explicitly considered in this assessment.

This draft screening assessment was prepared by staff in the CEPA Risk Assessment Program at Health Canada and Environment and Climate Change Canada and the Consumer Product Safety Directorate at Health Canada and incorporates input from other programs within these departments. The ecological portion of the assessment is based on the ERC-I document (published May 11, 2018), which was subject to an external peer review and a 60-day public comment period. The human health portion of

this assessment has undergone external peer review and/or consultation. Comments on the technical portions relevant to human health were received from Ms. Lopez, Ms. Super, and Ms. Jeney of Tetra Tech. Although external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

This draft screening assessment focuses on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA by examining scientific information and incorporating a weight of evidence approach and precaution.[2] This draft screening assessment presents the critical information and considerations on which the proposed conclusion is based.

2. Identity of substance

Talc (CAS RN[3] 14807-96-6) is one of the softest naturally occurring minerals, made up of magnesium, silicon, and oxygen (ChemIDplus 1993-). The term talc refers to both the pure mineral and a wide variety of soft, talc-containing rocks that are mined and used for a variety of applications (Kogel et al. 2006). Relatively pure talc ore is also referred to as steatite, and soapstone refers to impure, massive talc rock (Fiume et al. 2015).

The mineral talc is composed of triple-sheet crystalline units, consisting of two silicate sheets composed of SiO_4 tetrahedra joined by edge-link $MgO_4(OH)_2$ (Zazenski et al. 1995). These layers, held together loosely via van der Waals forces, slide over one another easily, giving talc its slippery feel and accounting for its softness (Fiume et al. 2015). The size of an individual talc platelet (i.e., a few thousand elementary sheets) can vary from approximately 1 µm to over 100 µm, depending on the conditions of formation of the deposit (Eurotalc 2017). The individual platelet size determines the lamellarity of a sample of talc. Highly lamellar talc will have large individual platelets, whereas microcrystalline talc will have small platelets. Other inorganics in place of magnesium and silicon are common in talc; for example, aluminum and iron may substitute for silicon in the tetrahedral sites, or manganese may substitute for magnesium in the octahedral positions (Zazenski et al. 1995).

[2] A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion on the basis of the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

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Commercially exploited talc contains 20 to 99 % of the pure mineral (Kogel et al. 2006). Some of the most common minerals that occur with talc are carbonates (e.g., dolomite, calcite, magnesite) and chlorite (i.e., magnesium aluminum silicate) (CIR 2013). Less common minerals include quartz, mica, iron oxides, pyrite, serpentine, and amphibole. Selective mining, ore processing, and beneficiation can remove many of the impurities (Kogel et al. 2006). There is a trend towards upgrading and higher-purity talc; however, many applications require the properties of the minerals associated with talc (Kogel et al. 2006). The purity of the source talc will influence its uses.

There are different grades of talc that refer to the purity (presence of other minerals). Pharmaceutical-grade talc conforms to the United States Pharmacopeia (USP) specifications (or similar specifications); these specifications require the absence of asbestos and set limits on iron, lead, calcium, and aluminum (USP 2011). As per B.01.045 of the *Food and Drug Regulations*, when used as a food additive talc must comply with Food Chemical Codex specifications or the Combined Compendium of Food Additive Specifications, prepared by the Joint FAO/WHO Expert Committee on Food Additives, and must be free from asbestos (FAO 2006).

Cosmetic-grade talc should comply with USP standards that require a limit of 20 ppm lead and an absence of asbestos (Fiume et al. 2015). Historically, some talc source materials were contaminated with asbestos; however, in 1976 the Cosmetic Toiletry Fragrance Association (CTFA) set purity standards for cosmetic-grade talc (Fiume et al. 2015). In Canada, the *Prohibition of Asbestos and Products Containing Asbestos Regulations* to be made under CEPA 1999 will prohibit asbestos above trace levels in consumer products, including cosmetics. Health effect studies on cosmetic-grade talc cited in this assessment were considered to be free of asbestos.

Talc is milled to different particle sizes for specific commercial applications. Most talc for cosmetics and pharmaceuticals are pure 200-mesh roller-milled talc (Kogel et al. 2006). In 200-mesh talc (preferred for body powder and deodorants), the particle size distribution allows 95 to 99 % of the product to pass through a 200-mesh (74 µm) screen (Zazenski et al. 1995; Kogel et al. 2006). The finer 325-mesh talc is also used in cosmetic-, pharmaceutical-, and food-grade formulations, where 95 to 99 % of the product passes through a 325-mesh (44 µm) screen.

3. Physical and chemical properties

A summary of physical and chemical properties of talc is presented in

Table 3-1. Talc is hydrophobic and lipophilic (Kogel et al. 2006).

Table 3-1. Experimental physical and chemical property values (at standard temperature) for talc

Property	Range	Key reference
Physical state	solid, powder	HSDB 2005
Melting point (°C)	1500	Eurotalc 2017
Vapour pressure (mm Hg)	approx. 0, negligible at 20°C	OSHA 1999; NIOSH 2014
Water solubility (mg/L)	insoluble	HSDB 2005
Specific gravity (unitless)	2.58–3.83	HSDB 2005

4. Sources and Uses

Talc is a naturally occurring mineral, and there are deposits of talc in most provinces of Canada (Kogel et al. 2006). Currently, there is one producing mine (open-pit) and concentrator facility in Canada, in Penhorwood Township near Timmins, Ontario, and one micronizing facility in Timmins (Kogel et al. 2006; MAC 2016; NPRI 2018). The talc ore from the mine is approximately 45 % pure, with magnesite, magnetite, chlorite, and serpentine as the major impurities (Kogel et al. 2006). After beneficiation, this mine and micronizing facility produces talc primarily for the paper, plastics, paint, and ceramic sectors (Kogel et al. 2006). In 2017, China was the largest producer of talc, followed by India, Brazil, Mexico, and Korea (USGS 2018). The major uses of talc globally include paper, plastics, paint, ceramics, putties, and cosmetics (USGS 2000; Kogel et al. 2006; EuroTalc 2017; USGS 2018) and are aligned with Canadian uses.

On the basis of information submitted pursuant to a CEPA section 71 survey for the year 2011, talc was reported to be manufactured and imported in Canada at quantities ranging from 50 to 75 million kg (EC 2013).[4] According to the Canadian International Merchandise Trade (CIMT) database, in 2016, 99 549 000 kg of natural steatite and talc, crushed or powdered (Harmonized System, HS code 252620) and 4 656 000 kg of natural steatite and talc, not crushed, not powdered (HS code 252610) were imported into Canada (CIMT 2017).

According to information reported pursuant to a CEPA section 71 survey, results from voluntary stakeholder engagement (ECCC, HC 2017), and a search of websites from talc producers, manufactured or imported talc is used in Canada in: adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials (e.g., wood and engineered wood); ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products,

[4] Values reflect quantities reported in response to the survey conducted under section 71 of CEPA (EC 2013). See survey for specific inclusions and exclusions (schedules 2 and 3).

mixtures, or manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment.

Talc is a formulant in pest control products registered in Canada (Health Canada 2010, Personal communication, email from the Pest Management Regulatory Agency, Health Canada to the Risk Management Bureau, Health Canada, dated March 29, 2017; unreferenced).

Additionally, in Canada talc is on the List of Permitted Food Additives with Other Accepted Uses for limited uses in a small number of foods (Health Canada [modified 2017]). Talc can be used as a coating agent on dried legumes and rice and as a filler and dusting powder for chewing gum as per the List of Permitted Food Additives with Other Accepted Uses, incorporated by reference into its respective Marketing Authorization issued under the *Food and Drugs Act*. It may be present in food packaging materials and in incidental additives[5] used in food processing establishments (email from the Food Directorate, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada, dated March 31, 2017; unreferenced).

Talc is present in approximately 8500 self-care products.[6] Talc is marketed or approved as a non-medicinal ingredient in approximately 1600 human and veterinary drug products in Canada, including approximately 150 over-the-counter (OTC) or non-prescription products (email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017; unreferenced). Talc is listed in the Natural Health Products Ingredients Database (NHPID [modified 2018]) with a medicinal role and classified as a natural health product (NHP) substance falling under item 7 (a mineral) of Schedule 1 to the *Natural Health Products Regulations* and with a non-medicinal role (NHPID [modified 2018]). Talc is listed in the Licensed Natural Health Products Database (LNHPD) as being present as a medicinal or non-medicinal ingredient, in currently licensed natural health products in Canada (LNHPD [modified 2018]). Talc is present as a medicinal or a non-medicinal ingredient in approximately 2000 active licensed NHPs. Talc is listed as a medicinal ingredient in diaper rash products in concentrations ranging from 45 to 100 % in the Diaper Rash Monograph (Heath Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]). Talc is permitted as a medicinal ingredient in the monograph for Traditional Chinese Medicine Ingredients (Health Canada 2015).

[5] While not defined under the Food and Drugs Act (FDA), incidental additives may be regarded, for administrative purposes, as those substances that are used in food processing plants and that may potentially become adventitious residues in foods (e.g., cleaners, sanitizers).

[6] Self-care products are products available for purchase without a prescription from a doctor, and fall into one of three broad categories: cosmetics, natural health products, and non-prescription drugs.

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, talc is an ingredient in approximately 6500 cosmetic products in Canada (dated April 5, 2017, emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). Talc is considered a restricted ingredient in cosmetics.[7] The Cosmetic Ingredient Hotlist entry for cosmetics containing talc in powder form intended to be used on infants and children indicates that product labels should display text to the effect of "keep out of the reach of children" and "keep powder away from child's face to avoid inhalation that can cause breathing problems." High-purity talc (fewer impurities of other minerals) is used in cosmetics, while lower-grade talc is used in the many commercial applications mentioned above. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics (Kogel et al. 2006; USGS 2018).

Condoms and medical gloves are regulated as Class II medical devices in Canada under the *Medical Devices Regulations* and may be sources of exposure if talc is present as a dry lubricant. However, a 1998 study did not find talc in a small survey of condoms tested in Canada (Douglas et al. 1998). Condom standards require dry lubricants to be bioabsorbable, such as starch and calcium carbonate (WHO, UNFPA, FHI 2013). Starch is more commonly used as dry powder lubricant on condoms (Douglas et al. 1998). There was also a shift from the use of talc as a dry lubricant on medical patient examination gloves to cornstarch in the 1980s (Lundberg et al. 1997). In 2016, the U.S. Food and Drug Administration banned powdered patient examination gloves (United States 2016).

5. Potential to cause ecological harm

5.1 Characterization of ecological risk

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I). The ERC-I is a risk-based approach that employs multiple metrics that consider both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past domestic and international assessment PNECs and water quality guidelines. When no suitable existing PNEC or water quality guideline was found, hazard endpoint data were collected and, dependent on data availability, either a species sensitivity distribution (SSD) or an assessment factor (AF) approach was taken to derive a new PNEC value. In the case of talc, hazard endpoint data from the Organisation for Economic Co-operation and Development

[7] Talc is described as a restricted ingredient on the List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances may contravene the general prohibition found in section 16 of the *Food and Drugs Act* (FDA), or may contravene one or more provisions of the *Cosmetic Regulations*. Section 16 of the FDA states that "no person shall sell any cosmetic that has in or on it any substance that may cause injury to the health of the user." In addition, the Hotlist includes certain substances that may make it unlikely for a product to be classified as a cosmetic under the FDA (Health Canada [modified 2018]).

Screening Information Dataset (SIDS) for synthetic amorphous silicates (OECD 2004) were identified for read across (ECCC, HC 2017) and an AF approach was used to derive a PNEC value of 40 mg/L.

Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. The generic near-field exposure model used input data, when available, from the National Pollutant Release Inventory (NPRI), the DSL–Inventory Update (DSL-IU), international trade data from the Canada Border Services Agency (CBSA), and third-party market research reports to generate PECs. In the case of talc, input data from the DSL-IU and CBSA were available.

Modelled PECs were compared to PNECs, and statistical metrics considering both the frequency and magnitude of exceedances were computed and compared to decision criteria to classify the potential for ecological risk as presented in ECCC (2018). The results are summarized in Table 5-1. The ERC-I identified talc as being of low ecological concern.

Table 5-1. Ecological risk classification of inorganics results for talc

Monitoring (total/extractable)	Monitoring (dissolved)	Modelling (DSL-IU)	Modelling (NPRI)	Modelling (CBSA)	Overall ERC-I score
NA	NA	Low	NA	Low	Low

Abbreviations: NA, Not Available.

6. Potential to cause harm to human health

6.1 Health effects assessment

Talc was previously reviewed internationally by the IARC, and an IARC monograph is available (IARC 2010). Additionally, talc was reviewed by the United States Environmental Protection Agency (U.S. EPA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany, and the Danish Environmental Protection Agency (Danish EPA) (U.S. EPA 1992; JECFA 2006; MAK-Commission 2012; Danish EPA 2016). Talc's safety in cosmetic uses was also assessed by the CIR Expert Panel (CIR 2013; Fiume et al. 2015).

A literature search was conducted from the year prior to the most recent assessment (the 2016 Danish EPA review), i.e., from January 2015 to January 2018. No health effects studies that could impact the non-cancer risk characterization (i.e., result in different critical endpoints or lower points of departure than those stated in existing reviews and assessments) for oral, dermal, or inhalation exposures were identified. For perineal exposures, recently published literature was identified and considered in the assessment.

The health effects of talc are outlined by route of exposure in the following sections.

Toxicokinetics

Talc is poorly absorbed via the oral route of exposure. Following gavage administration of radiolabelled talc to rodents, the majority of the administered dose (AD) remained in the gastrointestinal (GI) tract and was eliminated and recovered in the faeces (≥ 95.8 % of AD) within three to four days of dosing (Wehner et al. 1977a; Phillips et al. 1978). Less than 2 % of the AD was recovered in the urine; however, this was mainly attributed to contamination from faeces during collection, with true absorption and urinary clearance expected to be even lower. At 24 hours post administration, less than 2 % of the AD remained in the carcass of hamsters; no radioactivity was detected in mouse carcasses at this time point. In rats and guinea pigs, only trace amounts of radioactivity remained in the GI tract at 10 days post administration.

As an insoluble solid, talc is not expected to be absorbed when applied to healthy and intact skin. There are no indications of dermal absorption following talc exposure (MAK-Commission 2012).

Inhalable talc particles (<10 µm) are eliminated from the respiratory tract via mucociliary clearance. In female Syrian hamsters that were administered aerosolized neutron-activated cosmetic talc at concentrations of 40 to 75 mg/m^3 (95% pure; MMAD 6.4 to 6.9 µm) over a 2-hour exposure period, 6 to 8 % of the AD was deposited into the alveoli (Wehner et al. 1977b). The biological half-life following a single exposure was estimated to be between 7 and 10 days, with complete alveolar clearance after 4 months. There was no translocation of talc from the respiratory tract to the liver, kidneys, ovaries, or other parts of the body. Lung clearance was noted to be longer in other species. The Danish EPA (2016) noted that talc, including the respirable fraction (< 4 µm), is not absorbed following inhalation, but is retained in the lung tissue. They further stated that lung burdens were proportional to respired concentrations, and clearance became impaired with increasing exposures. Pulmonary retention half-lives for talc particles in the lungs of rats from a chronic inhalation study were estimated to be as long as 300 days (Oberdorster 1995). Other authors (Pickrell 1989; MAK-Commission 2012) noted similar findings indicating that with repeat exposures, alveolar clearance in rats may be impaired at concentrations of only 2 mg talc/m^3 air.

Talc particles have been observed and detected in the ovaries of humans (Heller et al. 1996a, 1996b), and perineal exposure to talc has also been associated with a presence of talc in lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996b; Cramer et al. 2007). Migration of talc particles from the vagina to the ovaries has been identified as a plausible explanation of these findings (Henderson et al., 1986), and retrograde movement of talc particles in humans through the reproductive tract to the ovaries has been suggested (Heller et al. 1996b; Cramer et al. 2007). Inert particles with the same size as talc (5 to 40 µm in diameter) and placed in the vagina can be transported to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979).

According to a review by the MAK-Commission (2012), there are no indications of metabolism via typical degradation pathways from which toxicologically relevant degradation products may develop.

Health Effects

Oral route of exposure

Talc was considered be of low concern with respect to human health via oral exposure. Repeated-dose testing with talc in animals did not produce any adverse effects via oral exposure with respect to repeated-dose toxicity, carcinogenicity, reproductive/developmental toxicity, or mutagenicity (Gibel et al. 1976; Wagner et al. 1977; NTP 1993; IARC 2010; Danish EPA 2016).

Talc has not been shown to produce adverse effects when ingested orally; as a result, the use of talc in various tablet formulations was not considered hazardous via the ingestion route (Hollinger 1990; U.S. EPA 1992).

In addition, the Commission of the European Communities' report on Dietary Food Additive Intake in the European Union identified talc as having an Acceptable Daily Intake (ADI) of "not-specified." The JECFA has also assessed talc and assigned an ADI as "not specified" due to the lack of toxicity from oral exposure. The substance was considered not to be a hazard to human health at oral intake levels noted in total diet surveys, which represent the majority of the sources of oral exposure for this substance (IARC 1987; EU [modified 2001]). Furthermore, talc is considered as "generally recognized as safe" when used as a food additive in the United States (U.S. FDA GRAS list) without being subject to pre-market approval requirements (U.S. FDA 2015; 2016).

Dermal route of exposure

There are limited data available on repeated-dose studies via dermal exposure to talc (Danish EPA 2016). In the available literature, only one repeated-dose dermal toxicity study was identified (Wadaan 2009). Severe limitations were noted for this study, including a lack of information on the test substance and the dose applied, as well as a lack of detail regarding the test animals. Skin dryness and erosion were noted; however, application sites were shaved, indicating that talc may have been applied to broken skin. As such, the results of this study were not considered appropriate to inform the characterization of health effects via dermal exposure. Additionally, there were no indications of irritation, sensitization, or dermal absorption following exposure to unabraded and/or non-diseased skin (MAK-Commission 2012). A three-day occlusive application of pharmaceutical-grade talc did not show any signs of irritation in 5 human volunteers (Frosch and Kligman 1976, as reported in MAK-Commission 2012).

Case reports, however, do indicate that the application of talc to diseased or broken skin can cause the formation of granulomas, particularly if the talc particles have a large diameter (MAK-Commission 2012; CIR 2013; Fiume et al. 2015). Granulomas have

been observed in the umbilical regions of infants, in the testes, on the vocal cords, in the urinary tract, and during phlebectomies following contact with talc-powdered surgical gloves (Ramlet 1991, Simsek et al. 1992, as reported in MAK-Commission 2012). As a result, the CIR concluded that "talc should not be used on skin where the epidermal barrier is removed or on skin that has greater than first degree burns."

Although dermal contact with talc is expected from the use of various products available to consumers, talc is a solid powder that is insoluble in water (Table 3-1). As a result, it cannot readily penetrate intact skin, and therefore systemic absorption through the skin is not expected. Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), a dermal health effects endpoint has not been identified for talc.

Inhalation route of exposure

Human studies

The Danish EPA (2016) noted that talc is not absorbed via inhalation. Rather, particles are retained in the lung, and lung burdens increase proportionally with exposure concentrations or frequency. The report detailed epidemiological data that noted mortalities in workers due to lung diseases, following exposures to talc. However, it was stated that there was no increase in the lung cancer rate in talc millers in the absence of exposure to carcinogens. A recent meta-analysis by Chang and colleagues (2017) reported a positive association with lung cancer in workers exposed to talc; however, co-exposure to other hazardous materials in the workplace and smoking were not adequately accounted for.

The chronic inhalation of talc leads to lung function disorders and fibrotic changes in humans. Since talc particles are persistent, particles accumulate in human lung tissue. This accumulation may lead to both an impairment of the self-purification function (reduced ability to fight infections) and inflammatory changes and fibrosis. Talc particles may be enclosed in a foreign-body granuloma as the result of an inflammatory reaction. The immobility of the macrophages, which is restricted by the phagocytized talc particles, leads to changes in the function of these cells and subsequently to chronic inflammatory reactions (Gibbs et al. 1992).

In humans, there are reports of pure talc-induced pneumoconiosis or talcosis following inhalation exposure to talc. Talcosis has been reported to occur in miners, millers, rubber workers, and other occupational groups exposed to talc without asbestos or silica (Vallyathan and Craighead 1981; Feigin 1986; Gibbs et al. 1992; Akira et al. 2007). Specifically, a recent longitudinal survey of French and Austrian talc workers found that the prevalence of small radiological opacities and decreases in lung function parameters were related to cumulative exposure. The mean estimated talc dust concentration during the mean duration of follow-up (14.5 years) was 1.46 mg/m^3 (Wild et al. 2008). Case reports indicate that patients present with non-specific complaints, including progressive exertional dyspnea, dry or productive cough, with indications of

lung lesions (Marchiori et al. 2010; Frank and Jorge 2011). Talcosis has been shown to occur in children and adults, with symptoms that developed shortly after acute to short-term exposure or up to 10 years later (Patarino et al. 2010; Shakoor et al. 2011). Inhalation of talc has been known to cause pulmonary effects, even following single acute exposures, as reported in a 10-year-old child who had a history of a single exposure to talc at two years of age (Cruthirds et al. 1977). Another case report detailed a seven-year-old child who developed asthma and reduced lung function after a single exposure event (Gould and Barnardo, 1972). Additionally, a 52-year-old woman who used baby talcum powder regularly at least twice a day (usually after bathing for personal hygiene and habitually applying it to her bed sheets nightly) for 20 years was reported to have dyspnea, along with a persistent dry cough and unintentional rapid weight loss. A radiographic exam noted evidence of interstitial lung disease with fibrosis (Frank and Jorge 2011).

Other relevant case reports include the case of a 55-year-old woman, occupationally exposed to talc as a dusting agent on packed rubber balls from 1958 to 1968, who was reported to develop dyspnea during the first five years after exposure (Tukiainen et al. 1984); and a 62-year-old woman occupationally exposed to talc for five years who was reported to have progressive lung fibrosis for more than 40 years (Gysbrechts et al. 1998).

Animal studies

In a repeated-exposure study conducted by the U.S. National Toxicology Program (NTP), groups of F334/N rats were exposed to aerosolized talc via the inhalation route of exposure. Test animals were exposed for 6 hours per day, 5 days per week, for up to 113 weeks (males) or up to 122 weeks (females) to aerosols of 0, 6, or 18 mg/m^3 talc (49 or 50 males per group, 50 females per group) (NTP 1993). Mean body weights of rats exposed to 18 mg/m^3 talc were slightly lower than those of controls after week 65. No clinical observations were attributed to talc exposure. Absolute and relative lung weights of male and female rats exposed to 18 mg/m^3 talc were significantly greater than those of controls. Inhalation exposure produced a spectrum of inflammatory, reparative, and proliferative processes in the lungs. Granulatomous inflammation, which was evident as early as 6 months (first histopathological examination), occurred in nearly all exposed rats, and the severity increased with exposure duration and concentration. Hyperplasia of the alveolar epithelium and interstitial fibrosis occurred in or near the foci of inflammation in many exposed rats, while squamous metaplasia of the alveolar epithelium and squamous cysts were also occasionally seen. Accumulations of macrophages (histiocytes), most containing talc particles, were found in the peribronchial lymphoid tissue of the lung and in the bronchial and mediastinal lymph nodes. In exposed male and female rats, there was a concentration-related impairment of respiratory function, beginning at 11 months, which increased in severity with increasing exposure duration. The impairment was characterized by reductions in lung volume (total lung capacity, vital capacity, and forced vital capacity), lung compliance, gas exchange efficiency (carbon monoxide diffusing capacity), and non-uniform intrapulmonary gas distribution (NTP 1993).

In female rats at 18 mg/m^3 talc, the incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) were significantly greater than those of controls (NTP 1993). The incidences of lung neoplasms in exposed male rats were similar to those in controls. Adrenal medulla pheochromocytomas (benign, malignant, or complex [combined]) occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m^3 talc groups were significantly greater than those of controls (NTP 1993).

The NTP (1993) concluded that there was some evidence of carcinogenic activity of talc in male rats on the basis of an increased incidence of benign or malignant pheochromocytomas of the adrenal gland. The NTP also concluded that there was clear evidence of carcinogenic activity of talc in female rats on the basis of increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland.

In a subsequent symposium, experts from the NTP, along with academic, industry, and government experts re-examined the results of the chronic inhalation studies. The general consensus from the expert panel was that the highest dose tested (18 mg/m^3) exceeded the Maximum Tolerated Dose (MTD) and as such, the neoplasms noted were not relevant to human health risk assessment (Carr 1995). A similar conclusion was rendered by Warheit et al. (2016). In addition, the Danish EPA (2016) and the MAK-Commission attributed lung tumours in female rats to the general particle effect of granular biopersistent dusts, which manifests as tumours in rodents only, and not the specific effect of the talc particles. They also attributed the pheochromocytomas to an increase in cell proliferation due to hypoxia, which was considered to be a high-dose effect (MAK-Commission, 2012).

A chronic, repeated-exposure study was conducted in B6C3F1 mice via the inhalation route of exposure (NTP 1993). Test animals were exposed for 6 hours per day, 5 days per week, for up to 104 weeks to aerosols of 0, 6, or 18 mg/m^3 talc (47 to 49 males per group, 48 to 50 females per group). Survival and final mean body weights of male and female mice exposed to talc were similar to those of the controls. There were no clinical findings attributed to talc exposure. Inhalation exposure of mice to talc at both concentrations was associated with chronic active inflammation and the accumulation of macrophages, which contained talc, in the lung. In contrast to rats, hyperplasia of the alveolar epithelium, squamous metaplasia, or interstitial fibrosis were not associated with the inflammatory response in mice, and the incidences of lung neoplasms in exposed and control groups of mice were similar. Accumulations of macrophages (histiocytes) containing talc particles were also present in the bronchial lymph node. The critical-effect level and corresponding health effects endpoint was a lowest observed adverse effect concentration (LOAEC) of 6 mg/m^3 for non-cancer lung effects (NTP 1993).

Doses used in the NTP chronic studies were selected on the basis of the results of a 4-week inhalation study (1993) in which rats and mice were exposed to talc at 0, 2, 6, or 18 mg/m^3, 6 hours a day, 5 days a week. Lung burdens were noted to be increased in a

dose-dependent manner, with overload noted by the study authors at 6 and 18 mg/m^3 in rats but not at any dose in mice. In both species (mice and rats), a minor macrophage infiltration of lung tissue was the only health effect noted in the high-dose animals, while animals in the mid- and low-dose groups were without treatment-related effects.

In a review of the NTP studies, Oberdorster (1995) revisited the lung deposition data and particle accumulation kinetics in the lungs of rats and mice in those studies, demonstrating that impaired clearance and lung overload was reached at 6 mg/m^3 and above, for both sexes, in rats and mice.

A no-observed adverse effect concentration (NOAEC) of 2 mg/m^3 was derived from the 4-week study, on the basis of increased lung burden and impaired clearance at a LOAEC of 6 mg/m^3 following 4-weeks of dosing, which led to non-cancer lung lesions at this concentration when the duration of dosing was extended. Granulatomous inflammation and alveolar epithelial hyperplasia were noted at a 6 month interim sacrifice in the chronic rat inhalation study, with interstitial fibrosis and impaired lung function noted in some animals at 11 months. As noted previously, following a single exposure in rats, the biological half-life for ciliary clearance was between 7 and 10 days, indicating that previous exposure would not have cleared prior to subsequent exposures, leading to a build-up in lung tissue. A re-examination of the NTP lung burden data by Oberdorster (1995) estimated that lung retention half-lives of talc particles were between 250 and 300 days in the rat chronic study. On the basis of this information, it was considered relevant to combine the NTP studies for the derivation of an appropriate point of departure for lung effects associated with repeated inhalation exposures.

The Danish EPA (2016) used the LOAEC of 6 mg/m^3 from the chronic NTP studies (mice and rats) and a NOAEC of 1.5 mg/m^3 for talc-induced non-cancer lung effects in the longitudinal survey of French and Austrian talc workers (Wild et al. 2008) to establish a health-based quality criterion for ambient air (QC_{air}) of 0.004 mg/m^3.[8]

While human occupational studies and case studies are available, these studies do not provide accurate measures of exposure for use in risk characterization. However, human studies do note a similar range of lung effects and disease as animal models. As such, results from the animal studies noted above were selected for the non-cancer risk characterization. On the basis of the NTP studies with rats and mice exposed to cosmetic-grade talc, a NOAEC of 2 mg/m^3 for non-cancer lung effects is considered to be appropriate for the inhalation route of exposure for short- or long-term use (given the long half-life and slow lung clearance of talc from the lungs, even episodic exposures would be expected to increase lung load). The NOAEC of 2 mg/m^3 was adjusted according to U.S. EPA guidance on inhalation risk assessment for a comparison with

[8] The health-based quality criterion in ambient air (QC_{air}) is a reference concentration that refers to the maximum permissible contribution to air from industrial sources.

exposure estimates (U.S. EPA 1994, 2009).[9] The adjusted NOAEC for non-cancer effects is 0.36 mg/m^3.

Perineal exposure to talc

The IARC has classified perineal use of talc-based body powder as "possibly carcinogenic to humans" (Group 2B) on the basis of limited evidence in humans. The analyzed case-control studies found a modest but consistent increase in risk, although bias and confounders could not be ruled out. The IARC Working Group concluded that, taken together, the epidemiological studies provide limited evidence in humans of an association between perineal use of talc-based body powder and an increased risk of ovarian cancer, although a minority of the Working Group considered the evidence inadequate because the exposure-response was inconsistent and the cohort analyzed did not support an association (IARC 2010).

The CIR Expert Panel (2013) determined that there is no causative relationship between cosmetic use of talc in the perineal area and ovarian cancer, and further concluded that talc is safe in the practices of use and concentration described in the CIR safety assessment. Issues noted by the CIR included a lack of consistent statistically significant positive associations across all studies; small risk ratio estimates; a failure to rule out other plausible explanations such as bias, confounders, and exposure misclassifications; and a lack of evidence from studies of occupational exposures and animal bioassays (CIR 2013; Fiume et al. 2015).

Animal studies

Rodents are poor experimental models for perineal studies for a number of reasons. Ovulation in rodents occurs only or mainly during the breeding season, and rodent ovaries are variously enclosed in an ovarian bursa in comparison to human ovaries. Ovarian epithelial tumours are also rare in these animals (Taher et al. 2018). Ovarian tumours do occur in some strains of mice and rats; however, the low incidence and/or the length of time required for the appearance of tumours renders them poorly feasible for experimental studies of ovarian carcinogenesis (Vanderhyden et al. 2003). On account of the limitations detailed above, in addition to the challenges posed by exposing animals via the perineal route, animal data are very limited; one single-dose study and one short-term repeated-dose study were available (Hamilton et al. 1984;

[9] This adjustment was made according to guidance and equations outlined in the U.S. EPA Supplemental Guidance for Inhalation Risk Assessment (US EPA 2009) and the U.S. EPA Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA 1994). Adjustment of duration to a continuous exposure scenario is done through the use of Equation 1 from U.S. EPA 2009 where the NOAEL[ADJ] = E × D × W, whereby the NOAEL[ADJ] (mg/m^3) = the no-observed adverse effect level (NOAEL) adjusted for the duration of the experimental regimen; E (mg/m^3) = the NOAEL or analogous exposure level observed in the experimental study; D (h/h) = the number of hours exposed/24 hours; and W (days/days) = the number of days of exposure/7 days. The NOAEC[ADJ] = 2 mg/m^3 × 6h/24h × 5d/7d = 0.36 mg/m^3

Keskin et al. 2009). No chronic or carcinogenicity animal studies on perineal exposure of talc were located in the literature.

A single injection of talc (in saline) into the bursa around the ovaries of rats showed foreign-body granulomas with confirmation of the presence of talc (Hamilton et al. 1984). Daily perineal or intravaginal application of talc (in saline) to rats for 3 months produced evidence of foreign-body reaction and infections; in addition, an increase in the number of inflammatory cells were found in all genital tissues. While no cancer or pre-cancer effects were observed, Keskin and colleagues (2009) noted that the study duration may have been too short to note these types of effects.

Human studies

Several meta-analyses of available epidemiological data have been published; some very recently (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). These studies have consistently reported a positive association with ovarian cancer and perineal talc exposure. Taher and colleagues (2018) identified 27 studies (24 case-control and 3 cohort) for a meta-analysis; ever versus never perineal use of talc and the risk of ovarian cancer resulted in a statistically significant pooled odds ratio (OR) of 1.28 (see Table 6-1). Other published meta-analyses have demonstrated similar results, with ORs ranging from 1.22 to 1.35 (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018).

Table 6-1. Available human epidemiological studies investigating the association of perineal use of talc and ovarian cancer (Taher et al. 2018, in preparation)

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
Case-control	686 (235)	Possible association in subgroup	Not included	Booth et al. 1989
Case-control	1014 (450)	Positive association	1.42 [1.08, 1.87]	Chang and Risch 1997
Case-control	336 (112)	Positive association in subgroup	Not included	Chen et al. 1992
Case-control	735 (313)	Positive association	1.60 [1.10, 2.33]	Cook et al. 1997
Case-control	430 (215)	Positive association	1.92 [1.27, 2.90]	Cramer et al. 1982
Case-control	4141 (2041)	Positive association	1.32 [1.15, 1.51]	Cramer et al. 2016
Case-control	3187 (1385)	Positive association	1.36 [1.14, 1.62]	Gates et al. 2008

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
Case-control	305 (153)	No association	2.49 [0.94, 6.60]	Godard et al. 1998
Case-control	1684 (824)	Positive association	1.30 [1.10, 1.54]	Green et al. 1997
Case-control	274 (116)	No association	1.10 [0.70, 1.73]	Harlow and Weiss 1989
Case-control	474 (235)	Positive association in subgroup	1.50 [1.00, 2.25]	Harlow et al. 1992
Case-control	306 (135)	No association	0.70 [0.40, 1.22]	Hartge et al. 1983
Case-control	2704 (902)	Positive association	1.40 [1.16, 1.69]	Kurta et al. 2012
Case-control	225 (46)	No association	1.15 [0.41, 3.23]	Langseth and Kjaerheim 2004
Case-control	3085 (1576)	Positive association in subgroup	1.17 [1.01, 1.36]	Merritt et al. 2008
Case-control	1354 (249)	Positive association in subgroup	1.37 [1.02, 1.84]	Mills et al. 2004
Case-control	2143 (1086)	No association	1.06 [0.85, 1.32]	Moorman et al. 2009
Case-control	2134 (767)	Positive association in subgroup	1.50 [1.10, 2.05]	Ness et al. 2000
Case-control	123 (77)	Possible association	1.00 [0.20, 5.00]	Rosenblatt et al. 1992
Case-control	2125 (812)	Possible association	1.27 [0.97, 1.66]	Rosenblatt et al. 2011
Case-control	1329 (584)	Positive association	1.44 [1.11, 1.87]	Schildkraut et al. 2016
Case-control	389 (189)	No association	1.05 [0.28, 3.94]	Tzonou et al. 1993
Case-control	727 (188)	Possible association	1.45 [0.81, 2.60]	Whittemore et al. 1988
Case-control	1155 (462)	No association	1.00 [0.80, 1.25]	Wong et al. 1999
Case-control	1297 (609)	Positive association	1.53 [1.13, 2.07]	Wu et al. 2009
Case-control	4092 (1701)	Positive association in	1.46 [1.27, 1.68]	Wu et al. 2015

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
		subgroup		
Cohort	108870 (797)	Possible association in subgroup	Not included	Gates et al. 2010
Cohort	78630 (307)	Possible association in subgroup	1.09 [0.86, 1.38]	Gertig et al. 2000
Cohort	41654 (154)	No association	0.73 [0.44, 1.21]	Gonzalez et al. 2016
Cohort	61285 (429)	No association	1.12 [0.92, 1.36]	Houghton et al. 2014

Abbreviation: CI, confidence interval.

Mode of action

The etiology of most ovarian tumours, in general, has not been well established. There are a number of different tumour types with characteristic histologic features, distinctive molecular signatures, and disease trajectories. Moreover, these tumours are heterogeneous, and they can arise from different tissues of the female reproductive tract, including the fallopian tube epithelium (National Academy of Sciences, Engineering, and Medicine 2016).

With respect to talc specifically, local chronic irritation leading to an inflammatory response is one possible mechanism of tumour progression that is frequently hypothesized (Muscat and Huncharek 2008; Penninkilampi and Eslick 2018; Taher et al. 2018). It is known that persistent indications of inflammation (including C-reactive protein, tumour necrosis factor, and other inflammatory markers) are detected in the blood of women prior to a diagnosis of ovarian tumours (Trabert et al. 2014). Increases in the number of inflammatory cells were found in all genital tissues of rats intravaginally exposed to talc for 3 months (Keskin et al. 2009). There is support for an association of inflammation and increased risk of ovarian cancer (National Academy of Sciences, Engineering and Medicine 2016; Rasmussen et al. 2017).

Talc particles were detected in the ovaries of rats that received intrauterine instillations of talc, and to a lesser extent in those that were dosed intravaginally with talc (Henderson et al. 1986). No translocation of talc into the ovaries was detected after single or multiple intravaginal applications of talc to rabbits (Phillips et al. 1978) or to monkeys (Wehner et al. 1986).

Talc particles were identified in 10 of 13 human ovarian tumours but were also found in 5 of 12 “normal” ovarian tissues removed from patients with breast cancer (Henderson et al. 1971). Ovaries from 24 patients undergoing incidental oophorectomy were examined; 12 women reported frequent perineal talc use, and the other 12 women were

non-users. Talc particles were detected in all 24 cases (both ever- and non-users) (Heller et al. 1996b). Wehner (2002) attributed the talc in the never users to (a) possible sample contamination, because some studies using negative controls resulted in particle counts similar to the test sample; and/or (b) possible false positives due to the use of a single radioactive tracer. To explain why talc is present in the never users, Heller and colleagues (1996b) hypothesized that talc use during diapering could contribute to the ovarian particle burden.

Translocation of other inert particles, similar in size to talc, has also been studied. A study in monkeys did not show any translocation of carbon black particles when a suspension was placed in the vaginal posterior fornix (Wehner et al. 1985). However, retrograde migration was detected when rabbits were administered a lubricant powder intravaginally (Edelstam et al. 1997). Other authors have noted similar transportation of particles to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979). There are also some indications that particles can migrate from the vagina to the upper reproductive tract in humans (Egli and Newton 1961; Venter and Iturralde 1979; Heller et al. 1996a,b), and perineal exposure to talc has also been associated with a presence of talc in the lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996a,b; Cramer et al. 2007).

Another possible mode of action that is hypothesized in the scientific literature is immune-mediated. It has been suggested that talc particles need not reach the ovaries but only need to reach the lower genital tract where talc could trigger changes (such as the production of heat shock proteins and/or decreased levels of antibodies) that could contribute to ovarian cancer (Cramer et al. 2005; Muscat et al. 2005). Human mucin 1 (MUC1) is expressed in high levels by ovarian cancer. Mucins are proteins involved in the formation of mucous barriers on epithelial surfaces (Gendler and Spicer 1995). Anti-MUC1 antibodies may have a protective effect; patients generate immunity against MUC1 produced by their tumours (Cramer et al. 2005). The Cramer et al. (2005) study used an enzyme-linked immunosorbent assay to measure anti-MUC1 antibody in women (controls; n = 721) to determine the factors that predict the presence of antibodies. It was found that the use of talc in the perineal area was associated with significantly decreased levels of antibodies to MUC1 (Cramer et al. 2005).

The most recent meta-analysis (Taher et al. 2018) employed the Hill criteria (Hill 1965) to assess the epidemiological evidence of a causal relationship. The Hill considerations are a set of factors (i.e., strength, consistency, specificity, temporality, biological gradient, biological plausibility, and coherence). These considerations form a framework for evaluating evidence in humans to help determine whether observed associations are causal (Hill 1965; Cogliano et al. 2004; US EPA 2005; Health Canada 2011; Fedak et al. 2015). Each factor, as reported in Taher et al. (2018), is elaborated upon below.

Strength: Of the 30 epidemiological studies examined by Taher et al. (2018), 15 case-control studies reported a positive association with statistical significance; 6 of these 15 had an OR of 1.5 or greater. Similarly, Penninkilampi and Eslick (2018) and Berge and colleagues (2018) each assessed 27 epidemiological studies and respectively

determined 14 and 13 case-control studies as reporting a positive association with statistical significance. In both cases, 5 of these studies had an OR of 1.5 or greater. Terry and colleagues (2013) only pooled 8 case-control studies; 5 of the 8 (63%) had a statistically significant positive association.

The individual cohort studies did not show a statistically significant association between perineal talc use and ovarian cancer (Berge et al 2018; Penninkilampi and Eslick 2018; Taher et al 2018). However, there was a positive association, with statistical significance, specific to invasive serous-type ovarian cancer in the cohort studies (OR = 1.25) (Penninkilampi and Eslick 2018). Given the long latency for ovarian cancer, the follow-up periods may not have been sufficient to capture all the cases for the individual cohort studies. Also, given the rarity of ovarian cancer, many of the available human studies may not be sufficiently powered to detect a low OR. Sample sizes were not large enough to detect a 20 to 30 % increase in risk; a group of over 200 000 women would need to be followed for over 10 years in order to detect a 20% (above background) increased risk with statistical significance (Narod 2016). With larger sample sizes, more individual studies may have demonstrated stronger associations.

Consistency: Several meta-analyses conducted over the past 15 years calculated similar ORs and resulted in similar conclusions; that there is a small yet consistent and statistically significant increased risk for ovarian cancer with perineal talc use (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al 2018). The epidemiological studies examined in these meta-analyses were conducted over different periods in time (across more than four decades), among different ethnicities, and spanned many geographical areas worldwide (Taher et al. 2018).

Specificity: Although there are many other risk factors for ovarian cancer (e.g., increased age, family history of cancer, obesity, nulliparity) (National Academy of Sciences, Engineering, and Medicine 2016), perineal talc exposure is specifically associated with cancer of the ovary and not other organs (Taher et al. 2018).

Temporality: In all case-control studies reporting positive outcomes, the participants recalled that exposure to talc preceded the reported outcome. However, in the cohort studies (reporting a lack of positive association), it is not known whether the follow-up period was adequate to detect a potential association between perineal talc exposure and ovarian cancer (Taher et al. 2018).

Biological gradient: There is a lack of an available exposure-effect relationship in the human epidemiological data. Many of the studies only assessed a single-dose level (ever versus never users). Furthermore, data with respect to the types of powder used by subjects or the amounts applied were not presented, and therefore a relationship between the concentration/dose of talc in the powder and the incidence of ovarian cancer could not be investigated. Taher and colleagues (2018) isolated seven studies that provided some evidence of increased risk of ovarian cancer with increasing perineal applications of talc; however, none demonstrated both a clear dose-response

trend and statistical significance (Whittemore et al. 1988; Harlow et al. 1992; Mills et al. 2004; Wu et al. 2009; Rosenblatt et al. 2011; Cramer et al. 2016; Schildkraut et al. 2016).

Biological plausibility: Particles of talc are hypothesized to migrate into the pelvis and ovarian tissue, causing irritation and inflammation. The presence of talc in the ovaries has been documented (Heller et al. 1996b). This evidence of retrograde transport supports the biologic plausibility of the association between perineal talc application and ovarian exposure; however, the specific mechanism(s) and cascade of molecular events by which talc might cause ovarian cancer have not been identified (Taher et al. 2018).

Coherence: Multiple case-control studies reported a lower risk of ovarian cancer in women who underwent pelvic surgery or tubal ligation (which disrupts the pathway and movement of talc from the lower to the upper genital tract) and suppressed ovulation (as cited by Taher et al. 2018: Cramer et al. 1982, 2016; Whittemore et al. 1988; Rosenblatt et al. 1992; Green et al. 1997; Wong et al. 1999; Mills et al. 2004). As noted in Penninkilampi and Eslick (2018), the main reductions in cancer incidence with tubal ligation were for serous and endometrial tumour types but not for mucinous or clear-cell tumours. Thus, tubal ligation is only effective in reducing the incidence of the same tumour types noted to be associated with perineal talc use.

The most recent meta-analysis detailed above (Taher et al. 2018), and consistent with the Hill criteria, suggests a small but consistent statistically significant positive association between ovarian cancer and perineal exposure to talc. Further, available data are indicative of a causal effect. A clear point of departure could not be derived from the available literature; consequently, hazard characterization is qualitative in nature.

6.2 Exposure assessment

This exposure assessment focuses on routes of exposure where critical effects have been identified; namely, non-cancer lung effects following inhalation of insoluble respirable particles of talc, and an association with ovarian cancer following perineal exposure to talc.

6.2.1 Environmental media, food and drinking water

Talc is a naturally occurring mineral, and there are several deposits in Canada (Kogel et al. 2006). Currently, there is one operating open-pit mine and concentrator along with an operating mill (MAC 2016); however, no talc concentration data in ambient air or around open-pit talc mines and processing facilities have been reported. Although particulate matter (PM) information for inhalable and respirable particles is available in the vicinity of these facilities (NPRI 2018), these data were not used in the exposure assessment as PM released from facilities is expected to contain a mixture of substances, hence the concentration would not reflect talc exposure from this source. However, given the

limited number of industrial and commercial sites producing and processing talc in Canada, talc exposure from ambient air is not expected to be significant.

Talc is insoluble in water (Table 3-1) and is expected to settle out during water treatment; exposure to the general population from drinking water is not expected.

There is potential for oral exposure resulting from the use of talc as a food additive; however, exposure from these uses is expected to be minimal (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the use of talc as a component in food packaging materials is expected to be negligible (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the oral route was not quantified because no critical health effects from the oral route of exposure have been identified. The JECFA has assigned an ADI of “not specified” for talc on the basis of low toxicity, and talc is “generally recognized as safe” as a food additive in the United States (JECFA 2006; U.S. FDA 2015).

6.2.2 Products available to consumers

Talc is present in approximately 8500 self-care products in Canada, including approximately 200 non-prescription drug products, approximately 2000 natural health products, and approximately 6500 cosmetic products. In addition, there are approximately 1300 prescription drugs containing talc. There is potential for oral exposure resulting from the use of self-care products and non-OTC drugs (including prescription, controlled substances, and ethical drugs) as a medicinal and non-medicinal ingredient containing talc. However, exposure from the oral route was not quantified as no critical health effects from the oral route of exposure have been identified.

There is the potential for dermal contact with talc from the use of self-care products. Systemic exposure resulting from dermal contact with talc is expected to be negligible as it is not expected that talc will be absorbed on the basis of its physical-chemical characteristics as an insoluble solid particle. In addition, a dermal health effect endpoint has not been identified for talc.

Notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, the LNHPD (modified 2018), the Drug Product Database (DPD), voluntary information submitted to Environment and Climate Change Canada and Health Canada (ECCC, HC 2017), publicly available databases and websites (e.g., Household Products Database 1993-; CPCat 2014; CPID 2017), and material safety and technical datasheets were used to identify products where there is: (a) the potential for inhalation of insoluble respirable talc, and (b) the potential exposure to the perineal region. These products and associated exposures are presented below.

No inhalation or perineal exposures were identified with respect to the major commercial or industrial uses of talc in paper, plastics, ceramics, and putties.

Inhalation exposure

For inhalation exposure, potential exposures were focused on products that were formulated as loose powders and were available to consumers, which included approximately 400 self-care products (primarily cosmetics). Products formulated as pressed powders, which comprise the majority of cosmetics containing talc (approximately 4000 products) were not identified as a potential source of exposure of concern because the formation of a “dust cloud” available for inhalation is not expected during the use of these products. Available information of interest were self-care products marketed as cosmetics, NHPs, or non-prescription drugs that are intended for application to the body, face, feet, buttocks (babies), and hair (e.g., dry hair shampoo). Concentrations of talc range from less than 10 to 100 % in these types of products.

In order to determine if talc loose-powder self-care products contain respirable particles, Health Canada measured the particle size distribution of three products (one baby powder and two adult body powder products) containing high concentrations of talc (>90%) available in Canada (Rasmussen 2017). Using an Aerodynamic Particle Sizer, the particle size distribution for the three products ranged from < 0.5 µm to 8 µm, with median particle sizes ranging from 1.7 to 2 µm. Thus, all of the particles were within the inhalable range (< 10 µm), and the median particle size was within the respirable range (< 4 µm). Number concentrations measured using a scanning mobility particle sizer indicated that the proportion of nano-sized particles (<100 nm) was small (< 10 %) to negligible, depending on the product.

Several studies were conducted by the cosmetic industry in the 1970s to provide data required to assess the safety of talc powder products and generate air concentrations (Aylott et al. 1979; Russell et al. 1979). These studies demonstrated that during the use of face, baby, and adult powders, there are quantifiable concentrations of respirable talc particles available for inhalation exposure. In 1978, Aylott and colleagues determined mean respirable air concentrations of 0.48 to 1.9 mg/m^3 of talc (< 7 µm) over 5 minutes for loose face powder, adult dusting powder, baby dusting powder, and micronized adult dusting powder. That same year, concentrations of talc (< 10 µm) of 0.19 mg/m^3 and 2.03 mg/m^3, respectively, were determined near the infant breathing zone during a simulation of routine application of talcum powder during diapering, and in the breathing zone of adults during the application of talcum powder to their body (Russell et al. 1979). In both of these studies, the highest air concentrations were associated with the adult application of talcum powder to their bodies over infant diapering and application of loose facial powder. There are uncertainties with the calculated talc concentrations determined from these studies due to limitations in the collection and analysis of talc concentrations on the basis of the use of older equipment, older sampling methods, and older talc products.

In 2017, a study assessing the health risk from the use of cosmetic talc from historical products was published (Anderson et al. 2017). This study included examining historical talc products from the 1960s and 1970s to characterize airborne respirable dust concentrations during the use of these products. To quantify respirable talc concentrations in the breathing zone, Anderson and colleagues (2017) designed a study where 5 volunteers were asked to apply historical talc products as they typically would in a bathroom setting. Cyclone air sampling devices were attached to the breathing zone of each volunteer. Each exposure simulation consisted of 8 application events, at six-minute intervals, for a total sampling duration of 48 minutes. This study design ensured that the sample mass on the sampling filter was large enough for quantification and accuracy, but it was not expected that during the typical use of a talc body powder that individuals apply talc every six minutes over a 48-minute window. Average talc concentrations over the 48-minute exposure simulation were calculated using the total measured mass (from 8 applications over 48 minutes) and the air volume over the entire 48-minute sampling period. Respirable talc concentrations ranged from 0.26 to 5.03 mg/m^3, and the average was 1.46 mg/m^3. The average air concentration by subject ranged from 0.44 to 3.28 mg/m^3. Respirable talc concentrations were more variable between subjects than within subjects, suggesting that individual behaviour has a strong influence in airborne concentrations.

In 2018, Health Canada conducted a small study in order to measure the air concentrations of particles in the breathing zone of adult volunteer subjects while they were applying talc-containing self-care products (Rasmussen 2018). Continuous, direct-reading, personal breathing-zone monitors (positioned beside the nose) measured average particulate matter of aerodynamic diameter of 4 µm or less (PM_4) concentrations of 0.48 ± 0.18 mg/m^3 and 1.80 ± 0.82 mg/m^3 for volunteers applying body powder and loose face powder, respectively. Subjects repeated the application in triplicate. These average concentrations fall within the range of concentrations measured by Anderson and colleagues (2017). In this study, the application of loose face powder resulted in the highest average air concentration in the immediate vicinity of the nose.

Several exposure scenarios were derived to characterize inhalation exposure to talc particles from the use of self-care products; namely, the use of baby, body, face, and foot powders (loose formulations), and dry hair shampoo. Average air concentrations by subject from Anderson et al. 2017 were combined with the body and face replicates from Rasmussen 2018 to obtain an overall average air concentration of 1.36 ± 0.97 mg/m^3. This value was used to estimate adjusted air concentrations for self-care products based on the highest concentration of talc present in these products. The results are summarized in Table 6-2. The inputs for each of these scenarios are outlined in Appendix A.

Table 6-2. Inhalation exposure estimates to talc from self-care products available to consumers

Product type	Age group	Concentration in air per event (mg/m^3)[a]	Adjusted exposure concentration (mg/m^3)[b]
Baby powder 100% talc	Infant and Adult	1.36	0.0071
Body powder 100% talc	Adult	1.36	0.0047
Face powder 100% talc	Adult	1.36	0.0047
Foot powder 97% talc	Adult	1.32	0.0034
Dry hair shampoo 100% talc	Adult	1.36	0.0011

[a] Average measured air concentrations (Anderson et al. 2017, Rasmussen 2018) × the highest concentration of talc in product type.
[b] Refer to Appendix A for details.

Perineal exposure

Several types of self-care products have the potential to result in exposure to the perineal region. There are several baby and body powders (approximately 50 products) with concentrations of talc that range from 0.3 to 100 %. There has been a decline in popularity of the use of talc for feminine hygiene practices over time; of 6000 North American women, 19 % of women born between 1920 and 1940 reported applying talc directly to the perineal region, but only 3% of women born after 1975 reported the same (Narod 2016). Houghton and colleagues (2014) reported that in 2001, the proportion of U.S. women who were users of perineal talc was estimated at 40 %, down from 52 % during 1993 to 1998.

There is a small number of diaper or rash cream self-care products (less than 10) which contains low concentrations of talc as a non-medicinal ingredient (up to 0.5 %). Talc is permitted as a medicinal ingredient in diaper rash products at concentrations from 45 to 100 % (Health Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]).

Additional self-care products that have the potential for perineal exposure (approximately 100 products) include antiperspirants and deodorants (e.g., genital antiperspirants), body wipes, bath bombs, and to a lesser extent (due to wash off or removal) other bath products (i.e., soap, shower gel) and products associated with hair removal (e.g., epilatory products). These products are formulated as gels, sprays, loose powders, and solid cakes, and range in concentration from less than 1% to 100% talc.

As indicated in Section 4, there is no evidence to suggest that talc is currently being used as a dry lubricant on condoms or medical examination gloves in Canada. At present, these are not considered to be sources of perineal exposure.

As a quantitative point of departure could not be derived from the available literature, perineal exposure from the use of self-care products was not quantified.

6.3 Characterization of risk to human health

Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), no critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and use of self-care products are not of concern.

Critical health effects have been identified following inhalation exposure to respirable talc particles. From the available toxicological studies, a NOAEC of 2 mg/m^3 from the NTP inhalation studies in mice and rats was identified in which non-cancer lung effects, with lung overload, were noted at the next highest concentration of 6 mg/m^3.

The average air concentration of talc following the use of a loose-powder self-care product (1.36 mg/m^3) provides a small margin of exposure (i.e., 1.5) to the NOAEC of 2 mg/m^3. However, the NOAEC is derived from a study with an exposure profile of 6 hours per day, 5 days per week, over 4 weeks, while the actual exposure scenarios from the use of self-care products are intermittent, occurring in minutes per day, daily, or weekly over many years. To address the differences in exposure between the NTP study and the actual use pattern, both the NOAEC and the talc air concentrations were adjusted to a continuous exposure scenario according to U.S. EPA guidance on inhalation risk assessment to more accurately characterize potential risk (U.S. EPA 1994, 2009). The NOAEC of 2 mg/m^3 is equivalent to an adjusted concentration of 0.36 mg/m^3, as noted in the Health Effects section. The NOAEC of 2 mg/m^3 was extracted from a 4-week inhalation study as a NOAEC for chronic exposure was not available. Episodic exposures from product use are expected to increase lung load due to the long alveolar clearance of talc. The adjusted air concentrations from the use of self-care products are presented in Table 6-3.

Table 6-3. Relevant exposure and hazard values for talc, and margins of exposure, for determination of risk

Exposure scenario	Adjusted air concentration, CA (mg/m^3)[a]	Adjusted critical-effect level (mg/m^3)	Critical health effect endpoint	MOE
Baby powder 100% talc	0.0071	NOAEC[adj]: 0.36	non-cancer lung effects	50

Body powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Face powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Foot powder 97% talc	0.0034	NOAEC[adj]: 0.36	non-cancer lung effects	106
Dry hair shampoo 100% talc	0.0011	NOAEC[adj]: 0.36	non-cancer lung effects	327

Abbreviations: adj, adjusted; CA, concentration in air per event; MOE, margin of exposure.
[a] From Anderson et al. (2017) and Rasmussen (2018), respectively, based on the highest concentration in products. For most of these product types, there is a wide range of talc concentrations (< 10 to 100 %).

The margins of exposure (MOEs) between the adjusted critical-effect level and the adjusted air concentrations range from 50 to 327 for self-care products. The MOEs for baby powder, body powder, face powder, and foot powder are considered potentially inadequate to account for uncertainties in the health effects (including a lack of a NOAEC from chronic studies) and exposure databases. The MOE for dry hair shampoo is considered adequate to address uncertainties in the health effects and exposure databases.

Based on available human data, ovarian cancer was also identified as a critical health effect for the perineal route of exposure to talc. There is the potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs). As noted in the Health Effects section, a point of departure cannot be derived for this health effect. Data from published meta-analyses of epidemiological studies indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). As noted by Narod (2016), “It is unlikely that the association between talc and ovarian cancer is due to confounding and so it is fair to say that if there is a statistically robust relationship between talc use and ovarian cancer it is likely to be causal.” Similarly, Penninkilampi and Eslick (2018) noted that “the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association.” Taher and colleagues (2018) noted that “consistent with previous evaluations by the International Agency for Research on Cancer (2010), and more recent and subsequent evaluations by individual investigators (Penninkilampi and Eslick 2018; Berge et al. 2018; Terry et al. 2013), the present comprehensive evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.”

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is the potential for perineal exposure to talc from the use of various self-care products, a potential concern for human health has been identified.

6.4 Uncertainties in evaluation of risk to human health

The inhalation of talc has been associated with a variety of non-cancerous lung effects, commonly termed talcosis. Dose-response data for lung effects in humans is, for the most part, lacking, and the use of animal data to quantify risk due to talc inhalation is considered appropriate. Despite the lack of exposure quantification, there are numerous case reports, as well as worker studies, that have identified non-cancer health effects from inhalation of talc powders. There is some uncertainty regarding the extrapolation of the NOAEC identified in animal models exposed for 6 hours per day for a short duration (4 weeks) to long-term episodic human exposures. The true NOAEC for chronic exposure is likely substantially lower than 2 mg/m^3.

Some self-care products, in particular, some face powders, may contain a cover or another mechanism that would reduce the potential for the generation of a particle or dust cloud, or that would reduce the concentration of the dust cloud during use of the product. There is uncertainty as to which products, and the proportion of products on the market, that incorporate these exposure-mitigation measures.

There are limitations with the human epidemiological data. Potential sources of bias include selection bias due to low response rates or from limiting subjects, and exposure misclassification due to recall bias (Taher et al. 2018). Muscat and Huncharek (2008) also proposed that symptoms of ovarian cancer prior to diagnosis may increase the perineal use of talc and bias the results. However, Narod (2016) and Berge and colleagues (2018) put less emphasis on recall bias. In studies where the exposure is simple (e.g., never versus ever use), recall bias is unlikely to be an important source of bias (Narod 2016). The positive association is strongest for the serous histologic type (Berge et al. 2018; Taher et al. 2018); findings that the association may vary by histologic type detracts from the hypothesis of report bias, as this type of bias would likely operate for all histologic types (Berge el al. 2018).

Ovarian cancer, in general, is not well understood (National Academy of Sciences, Engineering, and Medicine 2016), and a comparable animal model is not available. Health Canada has identified self-care products with the potential for perineal exposure (e.g., baby powder, body powders, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs); however, there is no indication exactly how the products are being used, the extent to which they would contribute to perineal exposure, and with what frequency and amount.

Talc use during diapering is a confounder that was not adequately accounted for in the epidemiological studies. It has not been determined whether the internal female genital

tract is exposed to talc dusts during infancy (Muscat and Huncharek 2008). As well, not all the available human studies are clear as to the formulations used for perineal applications. It is possible that the identified cancer incidences are specific to loose-powder formulations; however, there is inadequate information to attribute the cancer incidences to other formulation types (e.g., creams).

7. Conclusion

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(*a*) or (*b*) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(*c*) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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Appendix A. Inhalation exposure estimates

Table A-1. Estimated inhalation exposure concentrations from self-care products containing loose powder talc available to consumers

Scenario	Talc product conc.[a]	Study[b] conc. (mg/m^3)	CA[b] (mg/m^3)	ET[c] (hr/d)	EF[d] (d/yr)	ED[e] (yr)	EC adjusted (mg/m^3)[b]
Baby powder, infants	100 %	1.36	1.36	0.125	365	4	0.0071
Baby powder, adults	100 %	1.36	1.36	0.125	365	8	0.0071
Body powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Face powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Foot powder, adults	97 %	1.36	1.32	0.083	274	58	0.0034
Dry hair shampoo, adults	100 %	1.36	1.36	0.083	84	58	0.0011

Abbreviations: Conc., concentration; CA, concentration in air per event; ET, exposure time; EF, exposure frequency; ED, exposure duration; EC, adjusted exposure concentration.

[a] Highest concentration of talc found per product type from notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, DPD [modified 2018], email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; LNHPD [modified 2018], email from the Non-prescription and Natural Health Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; Fiume et al. 2015; Household Product Database 1993-; CPCat 2014; CPID 2017; SDS Search Tool 2016.

[b] Average by subject from Anderson et al. 2107 and Rasmussen 2018 (unpublished). CA = average study concentration × maximum talc concentration in product.

[c] ET is 5 minutes/application based on median time spent in the bathroom following a shower or bath (U.S. EPA 2011) × number of applications/day, whereby baby powder assumes 1.5 applications/day (CTFA 1983); the rest assume 1 application/day.

[d] EF is assumed to be daily for baby, body (U.S. EPA 2011) and face powder (Ficheux et al. 2015); foot powder 0.75 times/day or 274 times/year (Ficheux et al. 2015); dry hair shampoo 0.23 times/day or 84 times/year (Ficheux et al. 2015).

[e] Assumed infant wears diapers up to 4 years, adult exposure to baby powder from diapering children, 4 years per child and assume 2 children per family (Statistics Canada 2016), adult exposure for body powder, and foot powder (80 years lifetime, 12 years child).

[f] Adjusted exposure concentration is calculated as per Equation 8 in the U.S. EPA 2009 guidance document “Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual,” where EC = (CA × ET × EF × ED)/AT, and AT = averaging time, which is on the basis of ED × 365 days/year × 24 hours/day.